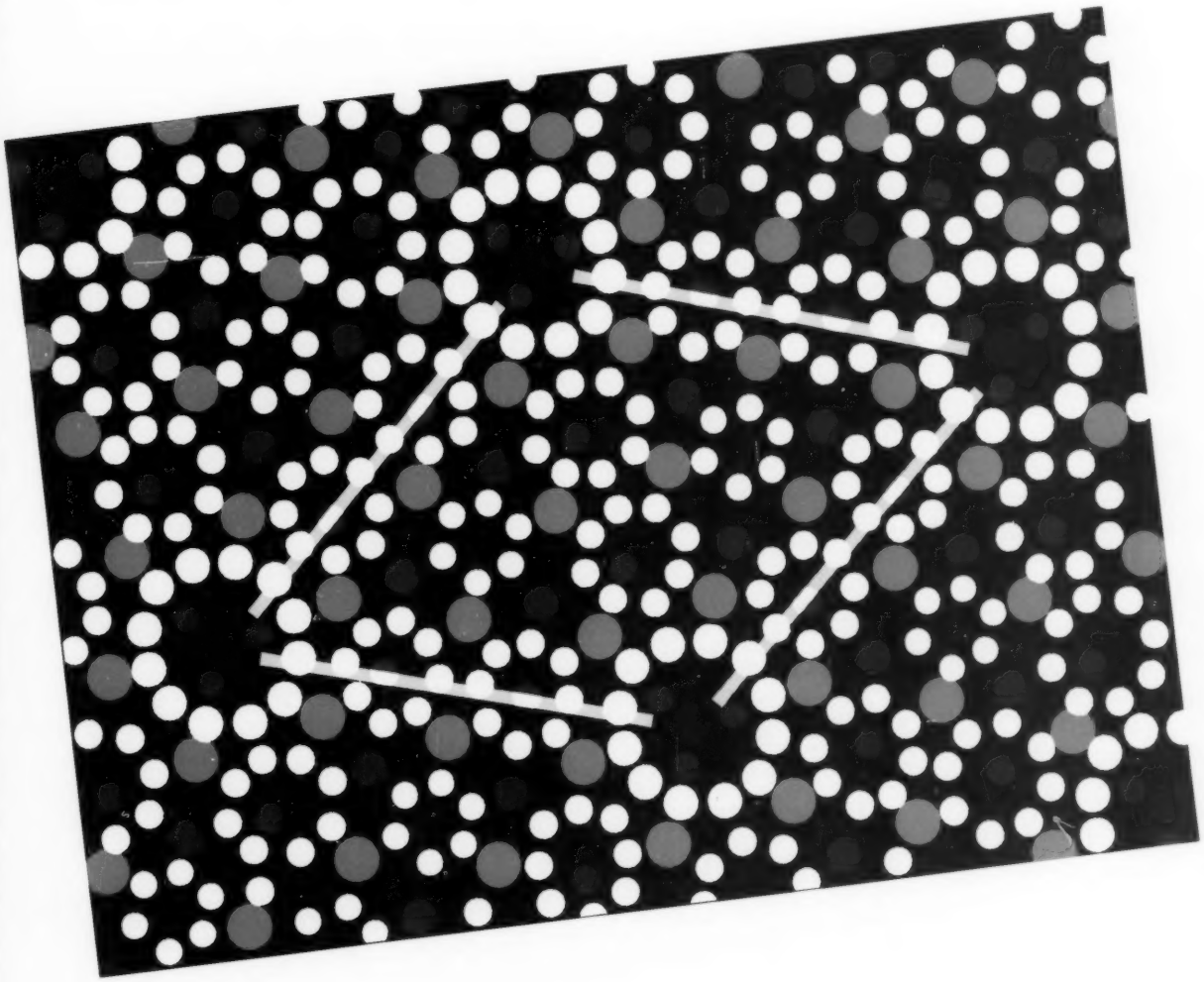
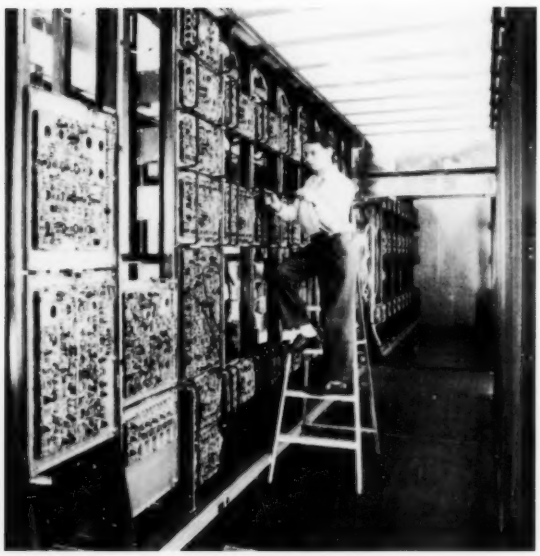
Naval Research Reviews

Office of Naval Research
Three / 1988
Vol XL

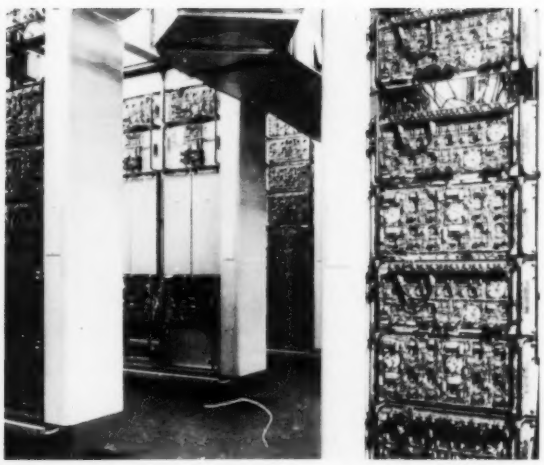


Project Whirlwind

Beginning in 1946 and continuing through the early 1950's, the Office of Naval Research sponsored the development of the first full-scale digital computer to utilize magnetic core memory. This research, which opened the way to the computer age, was accomplished under Project Whirlwind led by Jay W. Forrester at the Massachusetts Institute of Technology. Magnetic core memories resulted in more reliable and faster memories than both cathode ray tubes and delay line memories which were then available. Whirlwind remembered, acted upon, and delivered information at a rate of 20,000 times a second. Following this improvement, many computers were introduced utilizing random access magnetic core storage.



The Whirlwind computer filled a room when it was completed. This is a view of the finished computer from the entrance to the room.



A view of the arithmetic element of the Whirlwind computer.

Naval Research Reviews

Office of Naval Research
Three / 1988
Vol XL

Articles

2

Proximal Probes: Gateway to the Nanometer Scale World

by Richard Colton and James S. Murday

14

Theoretical and Experimental Studies Using a Planar Multifinger Manipulator

by J. Demmel, G. Lafferriere, J. Schwartz and M. Sharir

24

Molecular Biology of Marine Microorganisms: Biotechnological Approaches to Naval Problems

by Thoyd Melton and John W. Bodnar

Departments

13

Profiles in Science

40

Research Notes

41

Tech Base Notes

CHIEF OF NAVAL RESEARCH
RADM John R. Wilson, Jr., USN

DIRECTOR
Dr. Fred Saalfeld

DIRECTOR OF PUBLIC AFFAIRS
CDR Thomas E. Connor

CHIEF WRITER/EDITOR
William J. Lescure

SCIENTIFIC EDITORS
Dr. Robert J. Nowak
Dr. J. Dale Bultman
Dr. Albert W. Green
Mr. Steven W. Payne

MANAGING EDITOR
Marc J. Whetstone

ART DIRECTION
Typography and Design
Government Printing Office

About the Cover

Atomic structural model (top view) of the Si(111)- (7x7) by K. Takayanagi, Y. Tanishiro, M. Takahashi, and S. Takahashi, *J. Vac. Sci. Technol.* A3, 1502(1985). The unit cell is outlined by the diamond. The colored dots corresponds to the surface states associated with 19 dangling bonds (12 dangling bonds for the adatoms in the reconstructed surface layer (orange), 6 dangling bonds for the atoms in the top layer which are not bonded to the adatoms (light blue), and one dangling bond for the atom below the vacancy at the corner of the unit cell (dark blue)). Photo courtesy of J. Demuth, IBM Yorktown. See article on page 2.

Naval Research Reviews publishes articles about research conducted by the laboratories and contractors of the Office of Naval Research and describes important naval experimental activities. Manuscripts submitted for publication, correspondence concerning prospective articles, and changes of address, should be directed to Code OPARI, Office of Naval Research, Arlington, VA 22217-5000. Requests for subscriptions should be directed to the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20403. *Naval Research Reviews* is published from appropriated funds by authority of the Office of Naval Research in accordance with Navy Publications and Printing Regulations. NAVSO P-35.

PROXIMAL PROBES:

Gateway to the Nanometer Scale World

by Richard J. Colton and James S. Murday,
Chemistry Division
Naval Research Laboratory

Introduction

The publication of scanning tunneling microscopy images in 1982 (1) stimulated a revolution in surface and interface science. Binnig, Rohrer, Gerber and Weibel's (2) striking picture of atoms in the Si(111) 7×7 reconstructed surface (Figure 1) fired the imagination of the surface science community. At long last there was a viable approach to the problem of measuring surface structure.

But the imaging of surface atoms proved to be just a small part of the incipient revolution. In order to acquire the STM images, Binnig, Rohrer and their coworkers solved problems of vibration isolation and thermal drift; problems that had defeated previous efforts to position and control two bodies with Angstrom precision. While their initial approaches weren't simple (see apparatus on left in Figure 2), the successes awakened the general science community to the possibilities. The ensuring progress has been nothing short of spectacular.

A whole class of analytical tools, **Proximal Probes** (so named for the dependence on probe-sample proximity for their capabilities), is under creation. These tools can not only measure the composition/structure of surfaces with nanometer scale resolution, but also promise the capability to determine the chemical/physical properties of those structures. As an illustration of the progress made to date, the front cover shows the same Si(111) 7×7 surface as determined by later generation STM instruments (4) with diamond-shaped unit cell as an overlay. The colored dots highlight atoms which accept tunneling current at different tip-to-surface potential biases and correspond to surface states associated with 19 dangling bonds [12 dangling bonds for the adatoms in the reconstructed surface layer (orange), 6 dangling bonds for the atoms in the top layer which are not bonded to the adatoms (light blue), and one dangling bond for the atom below the vacancy at the corner of the unit cell (dark blue)].

The availability of analytical tools sensitive to nanometer scale structures provides compelling opportunities. First, these structures are a key to the science base of many venerable technological problems-corrosion, tribology, electrodes and adhesion most notably. These technologies have long histories of empirical progress (some dating back 4,000 years in recorded history), empirical largely because there has been no way of probing the phenomena on the scale believed critical—nanometer. Second, the nanometer scale also provides an opportunity to explore exciting new science concepts and novel material properties. For example neither atomic/molecular force laws, nor those developed for solid state, are accurate enough to predict the phenomena found at the nanometer scale. Nanometer size structures are smaller than critical scale lengths of many phenomena: far vs near-field electromagnetic radiation, superconductivity, ballistic electron transport, etc. Third, the possibility for fabricating three dimensional nanometer structures opens intriguing possibilities for electronic, magnetic and optical devices.

Fourth, the Proximal Probes will also stimulate improvements to other technologies. Precision engineering will improve with the augmented capability to measure at the nanometer level. Novel sensor concepts are starting to appear based on the tunneling tip as a highly sensitive technique for measuring displacements. New devices with very high density information storage are likely.

What exactly are these tools, the Proximal Probes, that generate so much promise and excitement? The next section will introduce you to some of them; the succeeding section will pique your imagination with some examples of their craft. By the end of this article, you should be convinced that, in contrast to present philosophies, the future is in doing things in a small way.

Figure 1

An early STM image of the Si(111) 7x7 reconstructed surface showing two diamond-shaped unit cells containing individual hills or bumps corresponding to 12 surface adatoms.

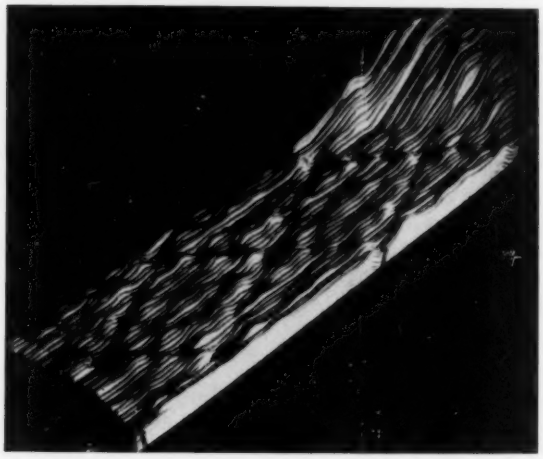
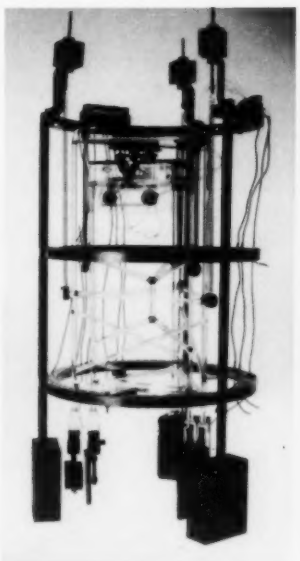
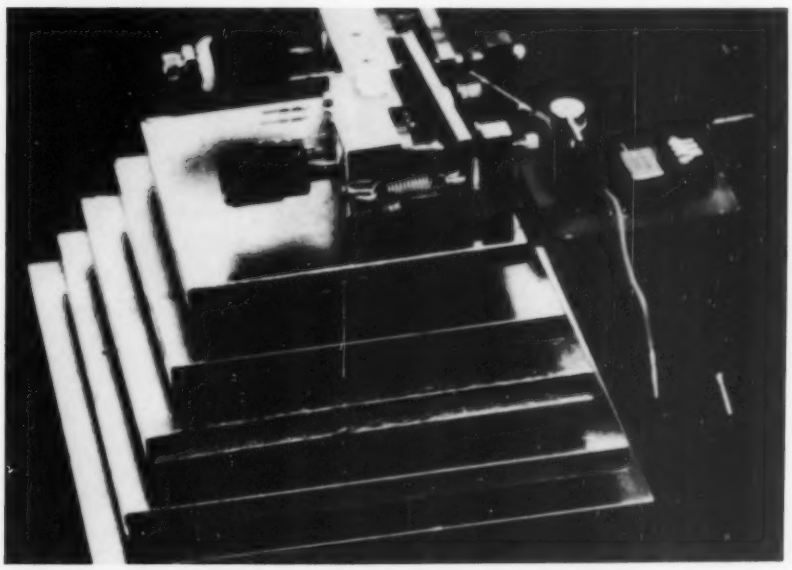


Figure 2

Schematic diagram of an older STM design (left) and a newer pocket-sized STM (right). The earlier design used an eddy current damped spring vibration isolation system while the newer (smaller) design uses a set of stacked plates separated by viton spacers.





Proximal Probes

Scanning Tunneling Microscopy/Spectroscopy (STM/S)

Scanning Tunneling Microscopy (STM) is based on the phenomenon that electrons, because of their wave-like nature, can tunnel through the potential barrier established when two electrodes (in this case a sharp tip and a conducting surface) are brought to less than a nanometer of each other (1-3). The concept of vacuum tunneling is quite simple; however, the experiment proved to be quite elusive, due primarily to the problems associated with maintaining a constant gap between two electrodes less than a nanometer apart. The solution of this problem by Binnig, Rohrer and co-workers at IBM Zurich in the early 1980's led to the development of STM and the 1986 Nobel prize in physics.

Electron tunneling and tunneling spectroscopy are actually mature disciplines in physics. There are many books (5-9) and papers devoted to the field. Tunneling through solid insulating barriers was first demonstrated in 1957. Early studies used what is known as "MIM" or metal-insulator-metal tunnel junctions which consist of a thin oxide layer between planar conducting electrodes. The thin insulating oxide layer defines a rigid - hence controllable - potential barrier through which electron could tunneling under appropriate potential bias. Examples of early MIM studies include semiconductor p-n junctions (10), the energy gap in superconductors (11), and molecular vibration spectra by inelastic electron tunneling (12). A modern day application can be found in Josephson-junction circuits (13).

Early examples of vacuum tunneling include electron emission from cold metals by a high external electric field—field emission—and the associated development of the field ion microscope (14) and the topografiner (15). Field emission was explained by Fowler and Nordheim by considering the one-dimensional tunneling problem (16). For small voltages, the tunneling current, I, can be expressed as

$$I \propto V \exp\{-A\phi^{1/2}s\} \qquad (1)$$

where V is the tunneling voltage, ϕ is the barrier height, s is the distance between the electrodes, and $A = 4\pi(2m)^{1/2}/h = 1.025\ eV^{-1/2}\ Å^{-1}$ when m is the mass of the free electron. Typically, when the work function of the metal is high (i.e., around 4 eV), the tunneling current increases one order of magnitude for 1 Å decrease in separation s. This exponential dependence of the tunneling current on distance gives STM its high sensitivity, but in addition requires the electrodes to be stabilized with better than 0.1 Å precision, particularly if atomic features are to be resolved.

In practice, then, the scanning tunneling microscope consists of a sharp tip that can be held less than a nanometer above a surface. Electrons tunnel between the tip and the surface when a small bias voltage is applied. An image of the surface can be acquired by scanning the tip across the surface. As the tip approaches a high (or low) spot on the specimen surface, the tunneling current begins to increase (or decrease) in response to the change in separation between the tip and the surface. In order to adjust the position of the tip to maintain a constant separation, a simple electronic feedback circuit is used to adjust the position of the tip to maintain a constant tunneling current. Scanning the tip in the plane of the surface produces a map or contour of the surface, much like a stylus profilometer. The power of the STM comes from its extremely high resolution—approaching 0.1 nm in the lateral dimension and 10^{-3} nm in the vertical direction which surpasses all other known techniques.

In addition to the imaging capability of STM, scanning tunneling spectroscopy (STS) is also readily done by measuring the current-voltage (I–V) spectrum. In metal-insulator-metal (MIM) tunnel junctions, where the tunnel junction is fixed by the thickness of the oxide layer, it is a simple matter to measure the current as a function of the applied voltage. In STS, however, varying the voltage under feedback control would cause the tip to reposition in order to maintain a constant current. There are two ways in which to prevent repositioning. One way is to sweep the voltage at a rate which is faster than the bandwidth of the feedback control circuit. The second way is to use a sample-and-hold feature that literally interrupts the feedtack loop long enough to sweep the voltage but short enough to leave the tip position stable.

The front cover again is an excellent spectroscopic example of the surface electronic structure of Si(111) 7×7. The ability to resolve spatially these surface states with the STM depends on a new method to acquire and separate geometric and electronic information. The method—current-imaging tunneling spectroscopy (CITS)—involves gating the feedback control circuit used for constant-current STM (4). When the feedback is active, a constant voltage is applied to the specimen; when the feedback is inactive, the position of the tip is stationary and the tunneling current is measured at various different bias voltages (i.e., I vs. V). The bias dependence of the images can not only be used to identify different surface energy or bonding states but may also be used to identify location of different elements on the surface. Another early example of this was given by Feenstra et al. (17) who used the STS to show the different positions of the Ga and As atoms in GaAs (110).

Atomic Force Microscopy

One telling limitation to STM/S is the necessity for transmitting nanoampere currents though very small areas; the substrate and tip need to be reasonably conducting. To remove this limitation Binnig came up with the concept of force microscopy (18) (Figure 3). Because the tunneling tip is very sensitive to small displacements, it can be used to detect sub-Angstrom deflections of a cantilever beam. If the beam is microfabricated or a fine wire, then very small forces are sufficient to bend it. When cantilevered and with a tip at the free end gently contacting by a surface, the beam bends; a tunneling tip sensitively measures the bend. Binnig et. al. (19) demonstrated that this approach to stylus profilometry could produce surface images with atomic resolution. Since then, several other groups have reproduced the result on graphite and extended the results to other lamellar compounds (20). Images of non-lamellar material surfaces have also been made, but with resolution nearer to 1-2 nm (21,22).

If the atomic force microscope tip is a conductor, then one can perform STM and AFM simultaneously (23,24). This has a powerful advantage. The tunneling tip measures displacements very sensitively, but does not provide an absolute calibration of the tip-surface separation. In particular, if an insulating film covers a surface, say an oxide on a metal, then tunneling current may not be acquired until the tip is poked through part of the film. Physical contact with the surface is readily detected by the AFM and can be used to benchmark the point of contact.

The atomic force microscope can be used to examine shear as well as normal forces. Mate et al.(25) have shown that friction coefficients can be measured with the AFM. In their work on graphite and mica surfaces, they have observed the very interesting behavior shown in Figure 4. Stick-slip phenomena are well known in tribology; contacting surfaces bind, freezing lateral motion until a force finally breaks the surfaces free. Just this kind of behavior has been observed with the AFM and lamellar substrates, except that the slip distances are multiples of the substrate unit cell. Whether the AFM stick-slip is in any way related to its macroscopic cousin is unknown. It is clear, however, that the AFM is a powerful new approach to the study of tribology, especially boundary lubrication and asperity contact.

Figure 3

Schematic diagram of an atomic force microscope in which a tunneling tip is used to measure the displacement of a soft cantilever beam as the sample, in contact with the free end of the cantilever, is scanned.

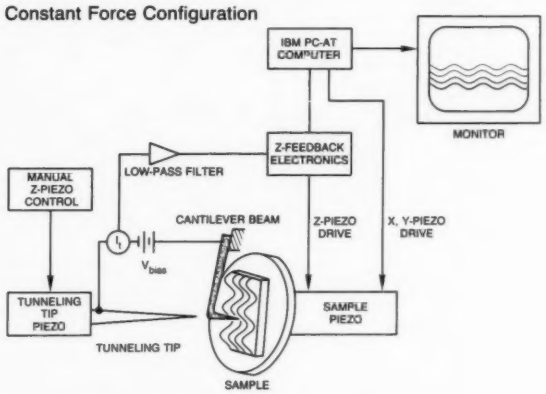
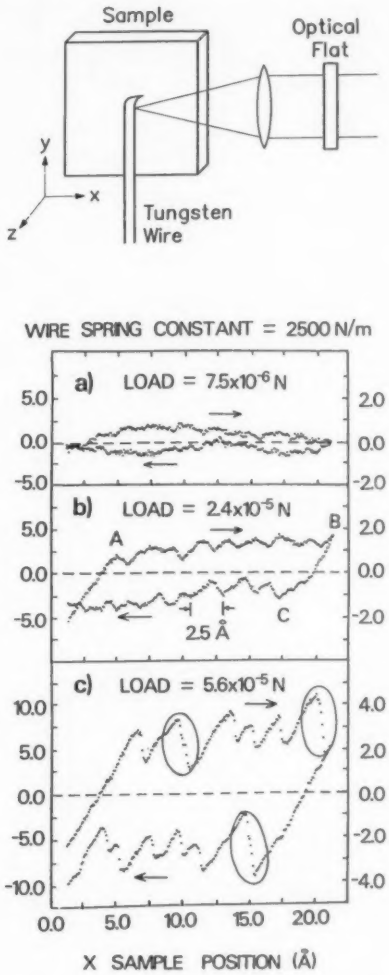


Figure 4

Schematic diagram of the atomic force microscope and optical detection system used to measure the shear or frictional forces of a tungsten wire sliding on graphite as a function of load.



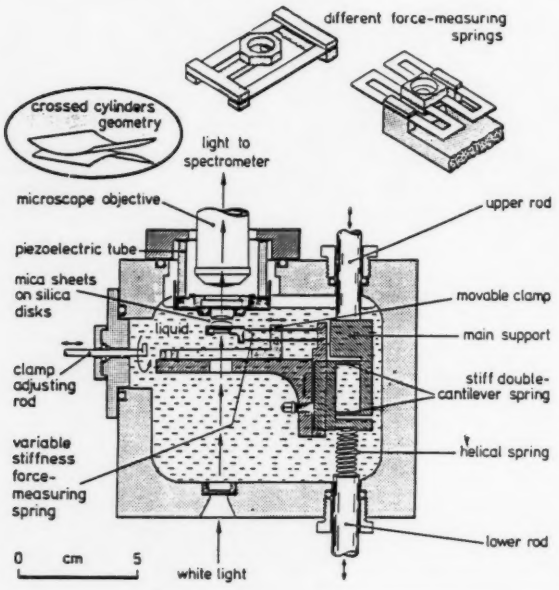
Force Microscopy/Surface Force Apparatus

The AFM detects the repulsive part of surface potentials (i.e. makes "contact"), it is also possible to detect small cantilever displacements caused by attractive forces between substrate and cantilever. Several variants of this approach have been demonstrated, including the mapping of magnetic structure (26,27) and surface potentials (28,29) with better than 10 nm resolution. This capability provides powerful new approaches to the investigation of magnetic films and integrated circuits, respectively.

There is a different sort of Proximal Probe, the surface force apparatus (SFA) (30) (see Figure 5) that has been developed independently from the tip based force microscopy. In this technique two crossed cylinders are brought close together. Optical interferometry is used to measure directly the separation of the two mating surfaces. One cylinder is cantilevered; contact forces between cylinders are measured by the amount it bends. The SFA variant does not allow for imaging with nanometer resolution. It does however provide independent measure of the force and separation, something that the tip-flat configuration of the force microscope does not allow.

Figure 5

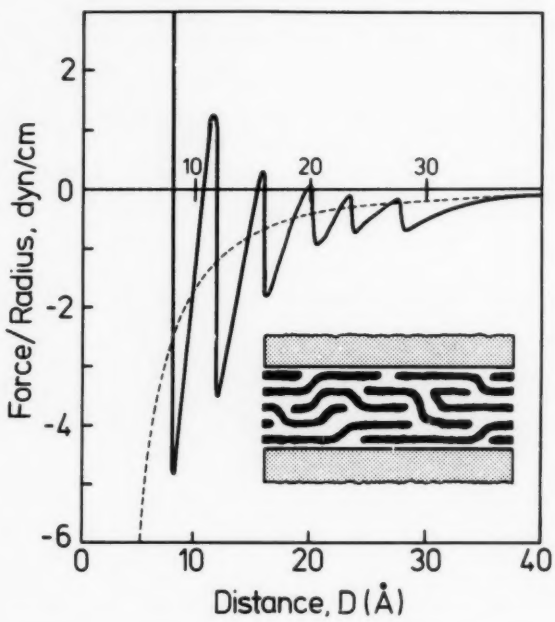
Schematic diagram of the surface force apparatus that employs the crossed cylinders geometry.



Results from the surface force apparatus have been equally startling as with the FM. For example, the attractive forces between two surfaces has been measured in vacuum and found to conform very well to classical dispersive force models. However, when liquid molecules are juxtaposed, striking differences occur (31); large oscillations appear in the surface forces (see Figure 6). The oscillations are presently attributed to structure in the "liquid" molecule boundary layers. The surface force apparatus has been modified to examine shear forces, also with tantalizing results (32). When the mating surfaces are within several "liquid" molecule diameters of each other, the friction coefficient force seems to have discrete values determined by the surface separation. Once again, structure in the boundary layer is invoked to explain the result.

Figure 6

Solvation forces measured with the surface force apparatus between mica surfaces in liquid tetradecane ($C_{14}H_{30}$) at 21 °C.

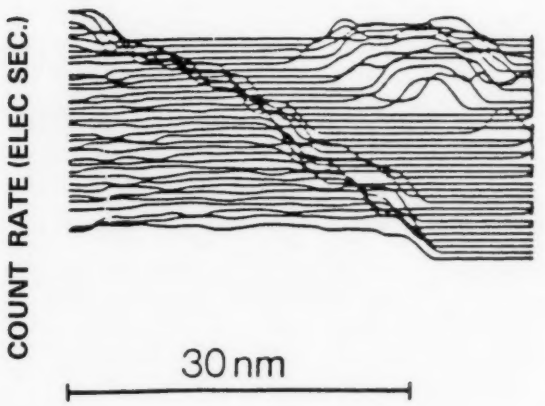


Field Emission Microscopy/Spectroscopy

The tunneling phenomena occurs only when the two surfaces are sufficiently close. As the surfaces are withdrawn to larger distances, the electron transfer occurs under the field emission approximation: emission from one surface into the vacuum, transport across the vacuum, and injection into the second surface. Work of McCord and Pease (33) and Fink (34) has shown that if the very sharp tips used in field ion microscopy are the electron source, the emission half angle is very small. Electron beams from these sharp tips placed close to the surface (~10nm) can have impact diameters of nanometer size. In the field emission mode, the potentials on the tip can be ~10–100V. This opens the possibility for proximal probe versions of several conventional analytical approaches–SEM, ELS, inverse photoemission, and Auger, for example. The line image in Figure 7 shows a 3 nm resolution SEM picture acquired with 15V electrons (34). Conventional SEM images would require >15kV electrons in order for the optics to focus the beam to this resolution. The lower energy electrons will be far more surface sensitive. Several demonstrations of spectroscopic studies have also been published (35,36).

Figure 7

Secondary electron micrograph of a polycrystalline gold sample showing features with a lateral resolution of ~3 nm. The image was generated with a 15 V electron beam from a tip positioned close to the sample surface.



Near-Field Electromagnetic Interactions

When two surfaces are brought closer than the wavelength of a photon, the normal far-field treatments of electromagnetic radiation are invalid. For instance, diffraction is a far-field phenomena; images can be obtained below the diffraction limit if a small aperture is located close (near-field) to the surface (37). Other novel phenomena also happen. The radiation transport of energy, described by the Stefan-Boltzman law, is a far-field phenomena. When surfaces are close (but not in contact) the energy flux may be considerably higher. The physics of this process is believed due to evanescent waves and may provide a new mechanism to map surface properties. Williams and Wickramasinghe (38) and Dransfeld (39) have shown that a microfabricated thermocouple tip can map local surface temperatures on surfaces.

Impact on Science/Technology

Analytical tools are a means to an end. Numerous phenomena of science and technology depend on the nanometer scale. Some of the more obvious (and important) are listed in Table 1. We will highlight several of these to demonstrate the power of the new Proximal Probe capabilities.

Surface Structure

The exquisite detail shown for the Si(111) 7×7 reconstructed surface has been illustrated on the cover. Covalently bonded surfaces are the best for STM characterization because of the directionality of their electron wavefunctions. The application of STM to electronic materials research has grown accordingly. But other materials are amenable as well. In Figure 8 the image of a conducting organic crystal TTF-TCNQ surface is shown (40) and compared to a calculated model of what the tunneling image should look like for the expected cleavage plane. There is little question that the STM has imaged the atoms on this organic crystal surface.

STM/S will also be a powerful tool for the study of molecular reactions on surfaces. Figure 9 shows the image of a benzene molecule coadsorbed with CO on the surface of rhodium (41). For the moment, the structure in the benzene images is not understood; it presumably reflects the electronic state of the molecule as modified by the metal substrate and/or the CO.

Table I:

Areas of Research Opportunities Stimulated by STM/AFM

Mesoscale Physics and Chemistry
surface structure—geometric & electronic
surface dynamics—diffusion & reaction
defect structure
phonon/vibrational spectroscopy
superconductivity
molecular imaging
solid/liquid interface studies
force laws

Nanometer Scale Electronics
lithograph
defect structure/surface states
interface studies
quantum devices
magnetic domain and domain wall

Materials Science
film nucleation & growth
composite interface
polymer structures
corrosion

Nanometer Scale Mechanics
polishing
tribology
elastic/plastic deformation
adhesion

Nanometer Scale Technology
position sensitive detectors
high density memory
magnetic recording
surface finishes

Biology
membrane structure
macromolecule configuration

Figure 8

a) STM image of the TFF-TCNQ surface showing an area of 2.5 x 2.5 nm². The arrows indicate a row of balls assigned to individual TCNQ molecules. b) Simulated image.

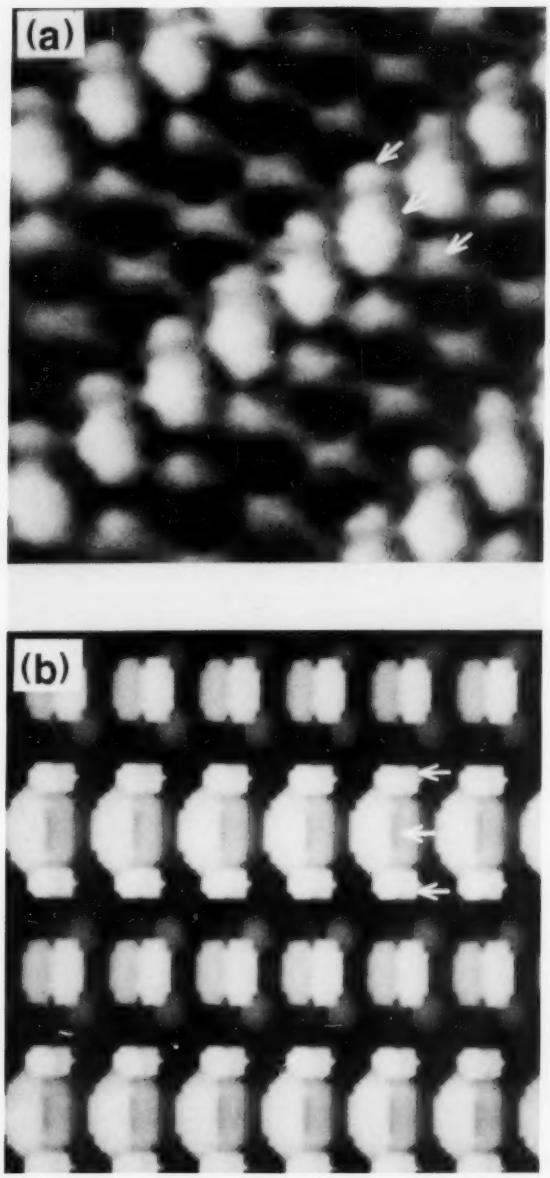
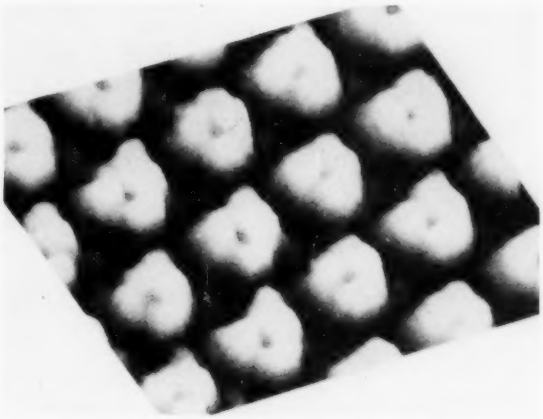


Figure 9

A three-dimensional STM images of benzene molecules on the Rh(111)–(3x3) (C_6H_6 + 2CO) surface. The lobes of the benzene molecule appear to be localized between, rather than over, the underlying metal atoms.



Boundary Layer Dynamics

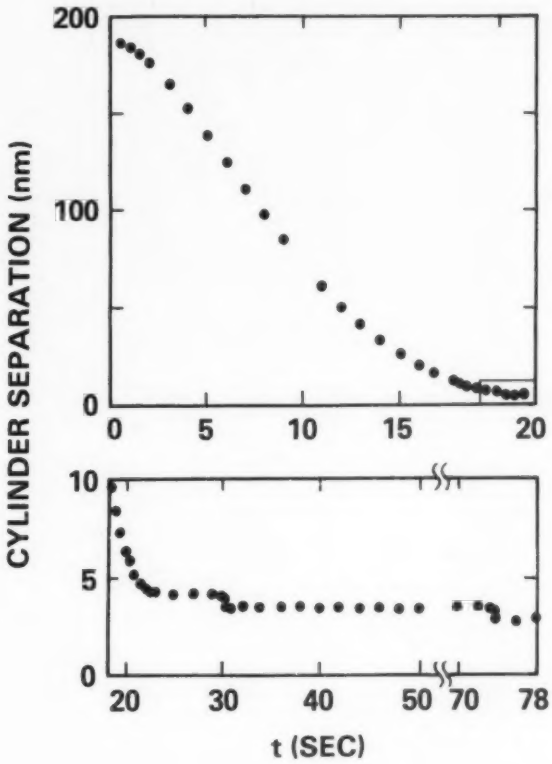
In addition to examining the steady state response to normal and shear forces, the surface force apparatus can be used to probe the dynamic behavior of fluids by instantaneously loading the cylinders and observing their separation as a function of time (42). When the cylinder separation is large compared to the dimensions of the fluid molecules, the cylinders close according to classical viscoelastic predictions. When the separation compares to molecular dimensions, the separation (see Figure 10) is constrained to discrete values, approximately integral multiples of the molecular size. The separation is metastable at each of those values before transitioning to the next. The tentative explanation of this behavior is that the fluid in the boundary layers is 'frozen' by the influence of the surfaces and that solid-state like diffusion processes are necessary to eliminate a layer.

Macromolecular Configurations

In the surface structure example above we showed the capability to image adsorbed simple molecules. What about the structure of more complex macromolecules where the molecular configurations reflect subtle energy differences? The ability to ascertain their surface structure would be very useful to biochemical and medical research. Figure 11 shows the image of a shadowed recA-DNA filament cluster (43). The shadowing was necessary to assist in the electronic conduction and to immobilize the molecule. More recent work, not yet published, indicates that DNA molecules may be imaged on a surface under water without any shadowing (44).

Figure 10

Measurement of the thickness as a function of time of a thin liquid film (octamethylcyclotetrasiloxane) as it is squeezed between two molecularly smooth mica surfaces. The drainage of fluid occurs in a series of abrupt steps whose size matches the thickness of molecular layers in the liquid.



Properties of Nanometer Structures

The properties of electronic devices will be dominated by interface phenomena as they grow smaller. We have seen how the structure of the semiconductor surface and adsorbed molecules can be imaged in detail. But adsorbed molecules can exchange charge with the surface, creating a localized space charge region. Can STM detect this localized perturbation? The images of 0 on GaAs shown in Figure 12 clearly show the space charge effects (45).

Interface structures, such as the adsorbed oxygen, can introduce charge trap sites which significantly affect device properties. Electrons can populate and depopulate these sites, introducing a source of scattering and noise. One STM study has been interpreted as measuring the rate of trapping/detrapping of a single surface defect site (28).

Figure 11

STM image of a shadowed recA-DNA filament cluster. The enhanced portions indicate the recA monomers.

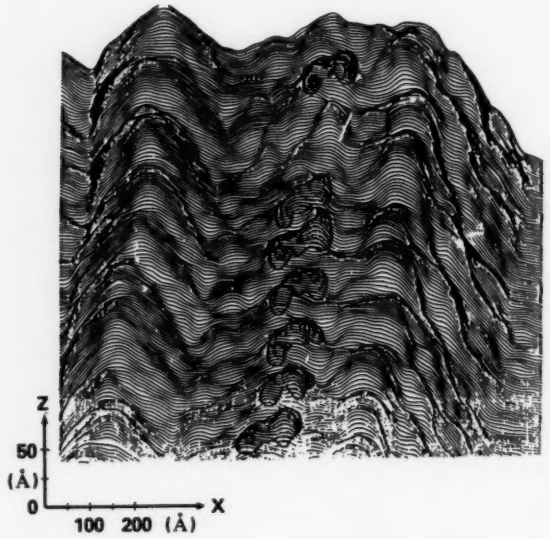
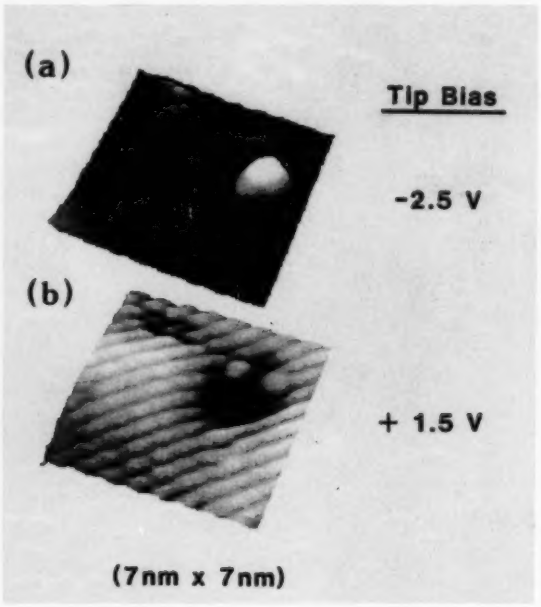


Figure 12

STM images of an oxygen defect on GaAs (110) acquired simultaneously at sample voltages a) −2.6 V and b) l.5 V.



Sensors

The tunneling tip can also be viewed as an exquisitely sensitive position detector. Displacements of 0.01nm are routinely reported, and more accurate measurements are postulated under special conditions. As a position sensitive detector, the tips have a unique advantage over other techniques. Both optical interferometry and capacitance techniques have the capability to measure equally small displacements. However these two approaches begin to lose sensitivity for structures smaller than a micron—optical interferometry because of diffraction limitations, capacitance because of S/N. The tunneling tip approach is limited only by the current that can be injected into an area without undue heat loading. Two device concepts have been published recently, a magnetic field sensor demonstrated to microgauss sensitivity (46) and an accelerometer demonstrated to $10\mu g/\sqrt{Hz}$ sensitivity (47). Temperature, pressure, electric field, gravity waves—any energy form capable of changing material dimensions should be detectable. Further, the tunneling process itself is affected by the presence of electromagnetic radiation; detection schemes for IR/visible radiation are being explored (48, 49).

Precision Engineering

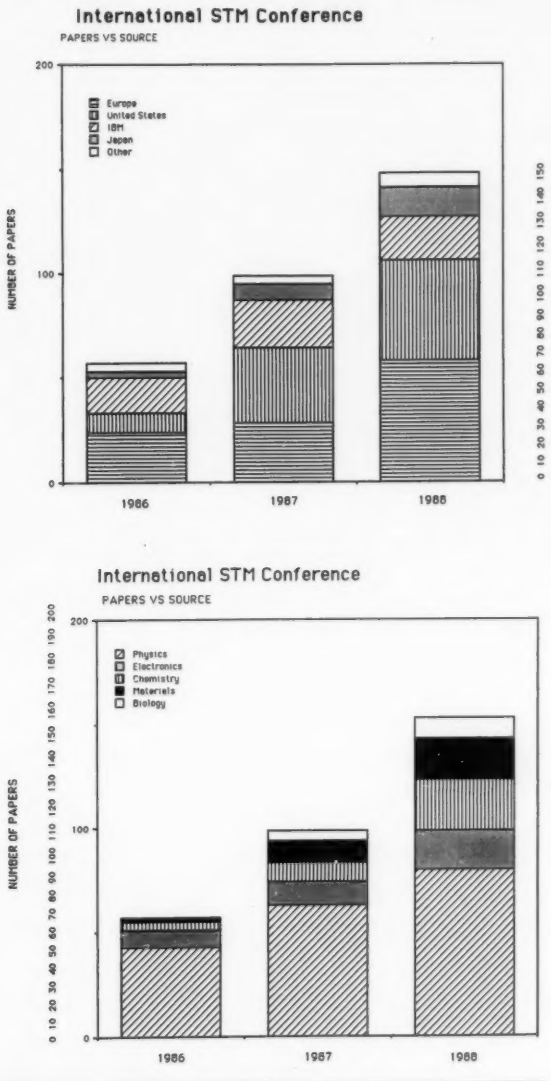
Higher energies, quieter systems, smaller parts—the demands of modern technology (especially the military) require performance characteristics which increasingly demand special interface properties. The evolution of the proximal probe techniques will provide the feedback necessary to engineer those interfaces. The late Richard Feynman once postulated a sequence of machines, each capable of fabricating a smaller machine, until the last was capable of working on the atomic level. We are now developing the tools to manipulate and measure properties on the atomic scale.

Conclusion

Progress in the nanometer world is accelerating at a breathtaking rate. Figure 13 shows that growth in the form of published papers, broken out both by point of origin and by discipline. The new class of analytical tool, Proximal Probes, enables that progress. The field is still very young, about five years old. It promises not only new insights into traditional technological problems but also fundamental challenges to our views of the chemistry/physics of matter. ONR and NRL is working hard to stimulate the progress for the benefit of science, the Navy, and the national economy.

Figure 13

Growth of STM/AFM and related areas in the form of publications broken out by: a) point of origin and b) discipline.



Biographies

Dr. Richard J. Colton is a research chemist and Head of the section on Advanced Surface Spectroscopy at the Naval Research Laboratory (NRL). He performed his graduate work under the direction of Professor J. W. Rabalais in the areas of ultraviolet and X-ray photoelectron spectroscopy. In 1976, he was awarded a National Research Council Resident Research Associateship at the Naval Research Laboratory for a research proposal dealing with secondary ion mass spectrometry (SIMS). His research interests include surface and materials analysis by electron spectroscopy and secondary ion mass spectrometry, the development of new surface analytical techniques, the study of the mechanisms of molecular and polyatomic ion emission, and most recently, the study of surfaces and molecular adsorbates by scanning tunneling microscopy.

Dr. James S. Murday is superintendent of the Chemistry Division at the Naval Research Laboratory, Washington, D.C., and consultant to the Surface/Interface Physics program of the Office of Naval Research. The Chemistry Division conducts R&D on nonmetallic materials, chemical dynamics, surface/interface science, combustion chemistry and biomolecular engineering. The ONR program emphasizes the solid-liquid interface, composite interfaces, and the fabrication/properties of nanometer scale structures. Presently he is serving on the American Vacuum Society Board of Directors and as an AVS representative to the American Institute of Physics Governing Board.

References

1. G. Binnig, H. Rohrer, Ch. Gerber, and E. Weibel, Phys. Rev. Lett. 49, 57(1982).

2. G. Binnig, H. Rohrer, Ch. Gerber, and E. Weibel, Phys. Rev. Lett. 50, 120 (1983).

3. G. Binnig, H. Rohrer, Ch. Gerber, and E. Weibel, Appl. Phys. Lett. 40, 178 (1982).

4. R. Hamers, R. Tromp, and J. Demuth, Phys. Rev. Lett. 56, 1972 (1986).

5. C. B. Duke, *Tunneling in Solids,* Academic Press, New York, NY, 1969.

6. *Tunneling Phenomena in Solids,* edited by E. Burstein and S. Lundquist, Plenum Press, New York, NY, 1969.

7. *Inelastic Electron Tunneling Spectroscopy,* edited by T. Wolfram, Springer Series in Solid-State Science, Vol. 4, Springer-Verlag, Berlin, 1978.

8. *Tunneling Spectroscopy,* edited by P. K. Hansma, Plenum Press, New York, NY, 1982.

9. E. L. Wolf, *Principles of Electron Tunneling Spectroscopy,* Oxford University Press, New York, NY, 1985.

10. L. Esaki, Phys. Rev. 109, 603 (1958).

11. I. Giaver, Phys. Rev. Lett. 5, 147 (1960).

12. R. C. Jaklevic and J. Lambe, Phys. Rev. Lett. 17, 1139 (1966).

13. D. G. McDonald, Phys. Today 34, 37 (1981).

14. E. W. Muller and T. T. Tsong, *Field Ion Microscopy, Principles and Applications,* Elsevier, New York, 1969.

15. R. D. Young, Phys. Today 21, 42 (1972).

16. R. H. Fowler and L. Nordheim, Proc. Roy. Soc. A119, 173 (1928).

17. R. M. Feenstra, J. A. Stroscio, J. Tersoff, and A. P. Fein, Phys. Rev. Lett. 58, 1192 (1987).

18. G. Binnig, C. F. Quate, and Ch. Gerber, Phys. Rev. Lett. 56, 930 (1986).

19. G. Binnig, Ch. Gerber, E. Stoll, T. R. Albrecht, and C. F. Quate, Surf. Sci. 189/190, 1 (1987).

20. T. R. Albrecht and C. F. Quate, J. Appl. Phys. 62, 2599 (1987).

21. O. Marti, B. Drake, and P. K. Hansma, Appl. Phys. Lett. 51, 484 (1987).

22. H. Heinzelmann, P. Grütter, E. Meyer, H. Hidber, L. Rosenthaler, M. Ringger, and H.J. Güntherodt, Surf. Sci. 189/190, 29 (1987).

23. U. Dürig, J. K. Gimzewski, and D. W. Pohl, Phys. Rev. Lett. 57, 2403 (1986).

24. P. J. Bryant, R. G. Miller, and R. Yang, Appl. Phys. Lett. 52, 2233 (1988).

25. C. M. Mate, G. M. McClelland, R. Erlandsson, and S. Chiang, Phys. Rev. Lett. *59,* 1942 (1987).

26. J. J. Saenz, N. Garica, P. Grütter, E. Meyer, H. Heinzelmann, L. Rosenthaler, H. R. Hidber, and H.J. Güntherodt, J. Appl. Phys. 62, 4293 (1987).

27. Y. Martin and H. K. Wickramasinghe, Appl. Phys. Lett. 50, 1455 (1987).

28. R. H. Koch and R. J. Hamers, Surf. Sci. 181, 333 (1987).

29. G. F. A. van de Walle, H. van Kempen, P. Wyder, and P. Davidsson, Surf. Sci. 181, 356 (1987).

30. J. N. Israelachvili, *Intermolecular and Surface Forces,* Academic Press, London, 1985.

31. J. N. Israelachvili, J. Coll. Interf. Sci. 110, 263 (1986).

32. J. N. Israelachvili, P. M. McGuiggan, and A. M. Homola, Science 240, 189 (1988).

33. M. A. McCord and R. F. W. Pease, J. Vac. Sci. Technol. B3, 198 (1985).

34. H.W. Fink, Physica Scr., in press.

35. B. Reihl and J. K. Gimzewski, Surf. Sci. 189/190, 36 (1987).

36. J. H. Coombs, B. Reihl, and J. K. Gimzewski, Proc. STM'88, to be published.

37. U. Durig, D. W. Pohl, and F. Rohner, J. Appl. Phys. 59, 3318 (1986).

38. C. C. Williams and H. K. Wickramasinghe, Appl. Phys. Lett. 49, 1587 (1986).

39. K. Dransfeld, Proc. STM'88, to be published.

40. T. Sleator and R. Tycko, Phys. Rev. Lett. 60, 1418 (1988).

41. H. Ohtani, R. J. Wilson, S. Chiang, and C. M. Mate, Phys. Rev. Lett. 60, 2398 (1988).

42. D. Y. C. Chan and R. G. Horn, J. Chem. Phys. 83, 5311 (1985).

43. G. Travaglini, H. Rohrer, M. Amrein, and H. Gross, Surf. Sci. 181, 380 (1987).

44. B. Barris, U. Knipping, S. M. Lindsay, L. Nagahara, and T. Thundat, Biopolymers, in press.

45. J. A. Stroscio, R. M. Feenstra, and A. P. Fein, Phys. Rev. Lett. 58, 1668 (1987); J. A. Stroscio and R. M. Feenstra, J. Vac. Sci. Technol. B6, 1472 (1988).

46. J. H. Wandass, J. S. Murday, and R. J. Colton, Sensors & Actuators, in press.

47. S. B. Waltman and W. J. Kaiser, Sensors & Actuators, in press.

48. A. A. Lucas, P. H. Cutler, T. E. Feuchtwang, T. T. Tsong, T. E. Sullivan, Y. Kuk, H. Nguyen, and P. J. Silverman, J. Vac. Sci. Technol. A6, 461 (1988).

49. L. Arnold, W. Krieger, and H. Walther, Appl. Phys. Lett. 51, 786 (1987) and J. Vac. Sci. Technol. A6, 466 (1988).

Profiles in Science

Dr. Rudolph A. Marcus is the Arthur Amos Noyes Professor of Chemistry at the California Institute of Technology. He is known for his work in many fields of theoretical chemical kinetics, including the theories of unimolecular reactions (RRKM theory), electron transfer reactions, electrode reactions, and various transfer reactions, the semiclassical theory of collisions and of bound states, and collision coordinates.

During the past 35 years, professor Marcus has been a principal investigator of the Office of Naval Research. The research projects for which he is best known and which have become standard theories used in chemistry (RRKM theory–"Marcus" theory of electron transfer reactions) were initiated under ONR support.

He is a member of the National Academy of Sciences, the American Academy of Arts and Sciences, and a foreign member of the Royal Society (London). Professor Marcus has served as Chairman of the Board of Trustees of the Gordon Research Conferences, and Chairman of the Division of Physical Chemistry of the American Chemical Society, member of the Executive Committee of the Division of Chemical Committee of the Western Section of the American Academy of Arts and Sciences. He has been a member of the Chemistry Review Committees of Brookhaven and Argonne National Laboratories, of the Notre Dame Radiation Laboratory, various university chemistry departments, the Advisory Board of the Petroleum Research Fund (ACS), the NSF Advisory Committee for Chemistry, and a variety of NAS-NRC Committees (Climatic Impact Panel on Atmospheric Chemistry, Committee on Chemical Sciences, Committee on Kinetics of Chemical Reactions (past Chairman), and Committee of Survey Opportunities in the Chemical Sciences).

He has been the recipient of an Alfred P. Sloan Foundation Fellowship, a National Science Foundation Senior Postdoctoral Fellowship, a senior Fulbright-Hayes Scholarship, the Anne Molson Prize in Chemistry of McGill University, the Chandler Medal of Columbia University, the Robinson Medal of the Faraday Division, Royal Society of Chemistry, the Irving Langmuir Award in Chemical Physics of the American Chemical Society, and the Wolf Prize in Chemistry. He has been awarded the honorary degrees of Doctor of Science by the Polytechnic University, by the University of Chicago, by the University of Goteborg, Sweden, and by McGill University. He was named the 1988 recipient of the A.C.S. Peter Debye Award in Physical Chemistry and of the Willard Gibbs Medal.

THEORETICAL AND EXPERIMENTAL STUDIES USING A PLANAR MULTIFINGER MANIPULATOR

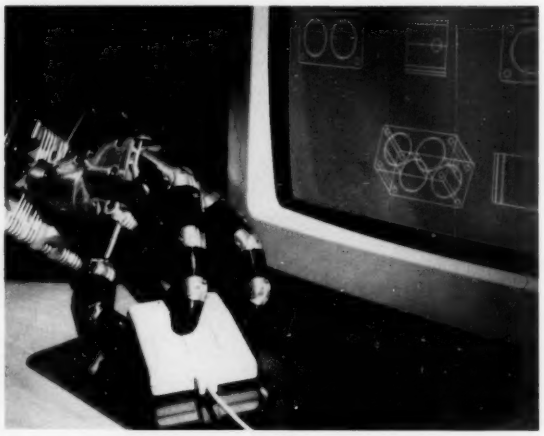
by J. Demmel, G. Lafferriere, J. Schwartz, M. Sharir,
New York University

Introduction

Dextrous manipulation, the use of robot hands to grasp and manipulate objects, has been the subject of much recent research activity. This paper describes work sponsored by the Robotics Program of the Office of Naval Research. One reason for this attention is the improvement in design and control of robot arms: Mechanical engineers have designed more accurate and more easily controlled arms, control engineers have designed faster and more stable control strategies, and computer scientists have produced better programming tools and computing engines to implement control schemes. (See the survey in [15].) These improvements have illuminated the fact that further flexibility and functionality in robots will have to come from more sophisticated grippers, or hands. While special purpose grippers have addressed some of the problems, they still do not permit maximum flexibility.

Two notable efforts to design general purpose robot hands led to the three-fingered Stanford/JPL hand [11], and the four-fingered Utah/MIT hand [7, 8]. These hands are much more complex than a single parallel jaw gripper. The Standard/JPL hand has three fingers with 3 degrees of freedom per finger, for a total of 9 degrees of freedom. Each finger is actuated by four flexible steel cables. Position and velocity information is obtained from incremental optical encoders located at the motor shafts. There are also 12 tendon tension sensors located at the base of the fingers. This hand was designed to maximize the common dextrous workspace of the fingers. The Utah/MIT hand has three fingers and an opposed thumb, arranged in an anthropomorphic configuration (see Figure 1). It has 4 joints (degrees of freedom) per finger (just like the human hand) for a total of 16 degrees of freedom. Each joint is actuated by 2 opposed flexible tendons (again like the human hand), for a total of 32 tendons. There are 16 joint angle sensors, one for each joint, and 32 tendon tension sensors, one for each tendon. An anthropomorphic configuration was chosen because the human hand is one of the few working examples of a truly general end effector. It is also possible to add tactile sensors to both hands, adding to the amount of sensor data. Other hand designs can be found in [5, 13].

Figure 1
The Utah/MIT Dextrous Hand.
(Photo courtesy of Photodynamics, N.Y.)



The price paid for the potential flexibility in these and similar dextrous hands is the complexity of dealing with so many degrees of freedom of motion and so many sensor readings (which are often quite noisy). This large number of degrees of freedom and large amount of data leads to the search for techniques to reduce the number of parameters to be controlled at one time.

A natural possibility is to use a hierarchical technique. At the lowest level of this hierarchy are the servo loops that control the actuators of the joints. Next come the controllers for each finger, then for groups of fingers and finally for the whole hand (and arm) system. Each level will take as input some form of the task specification and will output command specifications for the next lower level. This structure generates a hierarchy of research problems as well. At the lowest level we find the usual control engineering problem of designing and controlling joint actuators. At the finger level we find some of the traditional issues of robot manipulator control, where now the tip of the finger is the "end effector". However, the fingers usually do not have all 6 degrees of freedom, and they do not "grip" an object with their fingertips. Rather, it is the coordinated action of several fingers pressing at different points of the object that achieve the "grasp". So, at the next level we need to address the problem of how to coordinate the control of several fingers to manipulate a grasped object. It should be noticed that similar problems arise in the study of systems with closed kinematic chains such as multilegged locomotion systems (chains closed through the ground) and multiple manipulator systems (chains closed through the load). Here the distribution of forces among the gripping fingers (legs, arms) play an important role. Manipulation with several fingers usually involve modifying a grasp in the middle of a task with some fingers changing their gripping points or not taking part in the grasp altogether. Notice the difference with the typical robot arm where the tool or load is rigidly attached to some link of the robot through the end effector.

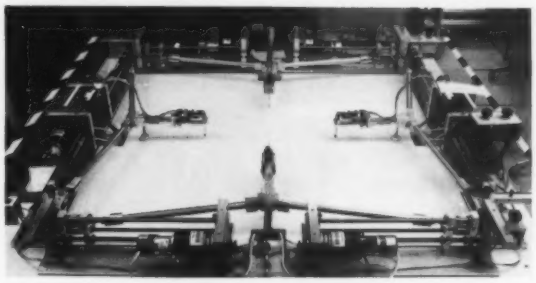
At the highest level there are the problems of task description and task planning. Here the issues are how to translate very high level instructions such as "hold knob and open door" or "pick up tool and trace edge" into a sequence of hand arm motions (including choosing a grasp) that will achieve the desired goals.

Compounded with all this is the fact that the more intelligent we want the robot to be the more we need to develop new sensors (with all the added complexity that they introduce in the control structure).

As this somewhat simplistic description suggests, there are many research problems to be solved before we can achieve truly general dextrous manipulation. In order to explore these issues in a simpler, more easily understood environment, we have designed and fabricated a simple manipulator called the Four Finger Manipulator (FFM) at the NYU Robotics Laboratory. The FFM has four independently controlled fingers which can move above a planar surface, for a total of 8 degrees of freedom (see Figure 2). In addition to position sensing, each finger has strain gauges which can measure the 2-dimensional force each finger tip exerts on its environment.

Figure 2

The NYU Four Finger Manipulator.
(Photo courtesy of Photodynamics, N.Y.)



Using the FFM, we tested our control algorithms by performing the following experimental tasks, which we will discuss in more detail later in the paper:

i. Hold the knob of a 2-dimensional 'door' and pull the door open (with and without external forces acting on the door). In this task, the position of the hinge with respect to the doorknob is unknown.

ii. Hold a 'tool' and follow a wall with it (keeping the tool at a prescribed angle to the wall), while exerting a constant normal force on the wall. The shape of the wall is unknown.

iii. Continuously rotate a disk by repositioning the fingers.

The rest of this paper is organized as follows: the FFM; our basic control approach, including the simplifying assumptions based on the special structure of the FFM; some details of the computation of force targets for the individual fingers, the core of the control loop; the measured results of experiments performed on the FFM (Our eventual goal is to use our experience gained in the 2-dimensional setting of the FFM and use it to design dextrous control schemes for the Utah/MIT Hand we have in our laboratory); and future work.

Description of FFM

The Four Finger Manipulator (FFM) is a simple two dimensional robot designed and fabricated at the NYU Robotics Laboratory to study dextrous manipulation and problems associated with real time control and manipulation. A schematic representation of the system (top view) is shown in Figure 3.

The FFM consists of four identical and independently controllable fingers that are mounted on the four sides of a square table (4th square). Each finger can be moved in the *xy*-plane with the aid of two stepper motors that are connected to the finger through a system of carriages and links. In all the robot has eight degrees of freedom (2 x 4 fingers). A finger tip (Figure 4) is mounted at the end of each finger. It consists of a vertical aluminum rod (2in long) with strain gauges mounted on its sides. The fingers grasp objects with the bottom end of the fingertips. The deflections of the aluminum rod are measured by the strain gauges, enabling the finger to sense a 2-*D* force acting on it. The accuracy of the strain gauges is about 0.01 Newton. Each of the eight stepper motors is controlled by an INTEL 8088 processor and has a position encoder attached to it. These processors, along with the other precision components provide a position accuracy of about 0.001 cm. Together, the strain gauges and motor encoders provide all the information required for force/position control of the system. The mounting table can be titled to perform experiments in the presence of gravity. The host computer for the FFM is a SUN workstation with a Motorola 68020 processor running the UNIX* operating system. This workstation serves as the primary system for software development, data storage, controller and data analysis. The FFM also has a lower level controller with a 68000 processor running the NRTX operating system. NRTX is a real time version of UNIX that provides several features for real time processing such as preemptive scheduling, multitasking, host controller communication etc. The lower level controller communicates with the finger motors and strain gauges. The architecture block diagram of the FFM system is shown in Figure 5. This architecture is rapidly changing in favor of a 68020 based system that will run the real time system SAGE developed by L. Salkind at NYU. A multiprocessor (NYU Ultracomputer [4]) is also being integrated into the system.

The FFM software consists of several levels: (1) a top level which controls and coordinates groups of fingers, (2) a group level which controls a single group of fingers, (3) a finger level, and (4) auxiliary code including safety check, calibration and archiving routines (for experiments data storage). The software structure was designed to match the hierarchy described in the introducion. This makes it simpler to develop new control algorithms for different tasks.

*UNIX is a Trademark of AT&T Bell Laboratories

Figure 3
Layout of the FFM.

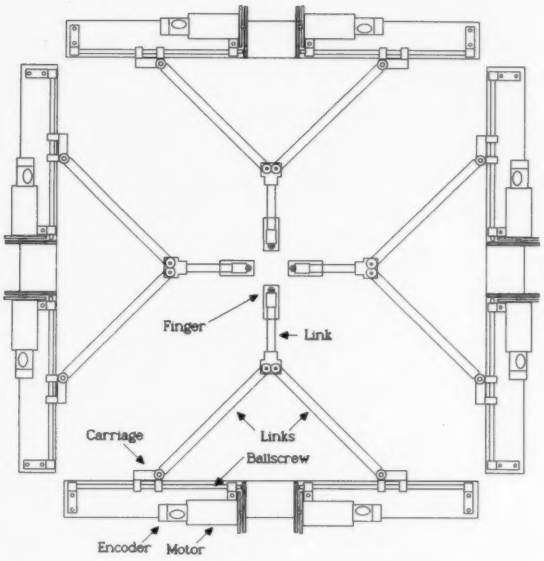


Figure 4
Finger Tip (side view.)

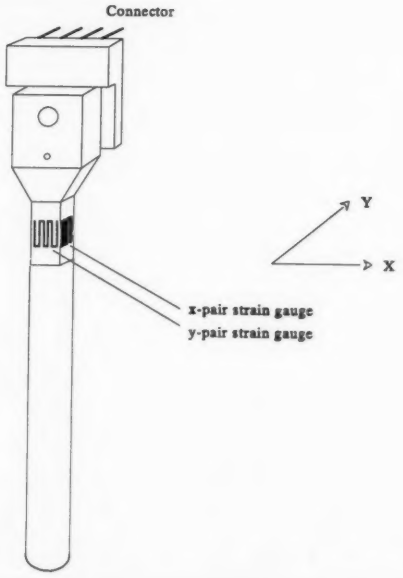
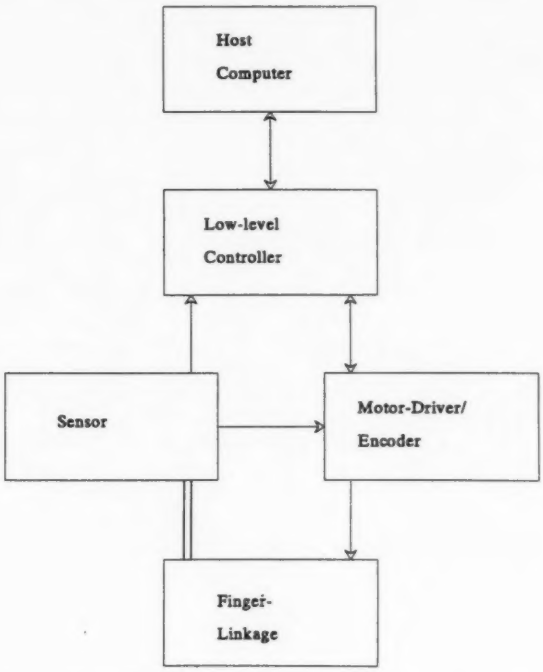


Figure 5
The Architecture Block Diagram of the FFM System.



Control Approach

In our early research effort we have concentrated on the second and third level of the hierarchy described in the introduction (fingers and groups of fingers). The analysis of control algorithms for these levels depends strongly on the fingertip model (hard, soft, point, round, with friction, etc.). Our initial model is that of a hard tip making point contact with the object. Furthermore, we assume there is friction between the finger and the object. We developed a closed loop control strategy dealing with the distribution of forces among several independently controlled fingers manipulating an object being held in a fixed grip. Our approach treats the grasped object as a *virtual finger* allowing forces on and positions of the object to determine positions and forces for the individual fingers. The transformations involved are linear and can be computed once the grip is established and need not be recomputed unless the gripping points change. That is, we can set target positions and forces for the object depending on the task to be accomplished and by applying the appropriate transformations obtain the targets for the individual fingers.

The *gripping forces*, which depend on the coefficients of friction at the gripping points, are chosen by defining some suitable point of concurrence for the finger forces.

Our control approach involves the following phases:

(A) Given a task to be performed, an appropriate combination of force and position targets for the object is determined. These depend on the force that the object is supposed to exert on its environment and the motions it is supposed to undergo during a manipulation. Furthermore, this phase will also include the decomposition of the task into *homogeneous manipulations* (i.e. manipulations which can be achieved using a single "smooth" control law). This phase takes place before the fingers perform any motion.

(B) For each homogeneous manipulation and based on the gripping positions and constraints a linear transformation is defined that maps the force/position targets of Phase A to force/position targets for each of the fingers. This phase is computationally intensive. It is carried out after the fingers have gripped the object but before each manipulation takes place.

(C) Finally there is an inner *feedback control loop.* This phase is run in real time.

The various types of object targets arising in Phase A are illustrated by the following manipulation tasks.
(T1) Use several fingers to grip a freely movable object *O*, and then move *O* along a specified path while applying grip forces of user-specified intensity.

(T2) Apply grip on an object *O* lying on a table-top, and exert force sufficient to overcome gravity (here note that the weight and center of gravity of *O* will generally not be known in advance).

(T3) Grip an object *O* constrained by various hinges, pegs and slots, and move it along the unique path available to it.

(T4) Grip the handle of a spring loaded crank and turn it.

(T5) Rotate an object continuously by repositioning the grasping fingers.

In (T1) we hold the finger forces constant (relative to the gripped object). In (T2), we *overcome* all external forces and at the same time adjust the *gripping forces* so as to avoid slippage. In (T3) we are *guided by* external forces by either keeping constraint-generated forces as low as possible (door opening) or having them maintain constant positive values (wall following). For (T4) we want to be *guided by* external forces in some directions while *overcoming* the external forces in others. Specifically, we are guided by the component of the external force which is normal to the trajectory of the crank knob while we overcome any tangential force generated by the spring. For (T5) a new hierarchy of control is needed to direct one finger to reposition itself while the others are rotating the object.

Computation of Finger Forces

We give the details for Phase B in the case of 3 fingers moving on a plane (which is the case of the FFM). Let $F=(F_1, F_2, F_3)$ be the vector of finger forces. Given the desired object force/torque target $w=(E,\tau)$ we need to solve

$$\sum_{i=1}^{3} F_i = E, \qquad \sum_{i=1}^{3} F_i{}^T \bar{r}_i = \tau \tag{1}$$

where r_i is the vector from the nominal center of torque to the position of the i^{th} finger, and $\bar{x}$ designates the vector obtained from x by rotating 90° counterclockwise. This can be written as $G(F)=w$. The solution to (1) can be written as G^+w+e where $G^+=G^T(GG^T)^{-1}$ is the pseudo inverse of G and e is a solution of the homogeneous equation (i.e. a vector of purely gripping forces). Since in this case G is 6×9 and has rank 6, the null space of G has dimension 3. This freedom can be interpreted as coming from the two dimensions of the point of concurrence of the gripping forces and from the *tightness* of the grip. We proceed now to find the gripping forces by choosing the point of concurrence which minimizes the dependence of the grip on friction. More precisely, we find e as follows. We find the set of points p such that all the lines through p and the gripping points make equal angles θ with the corresponding normals. Then, we select the point for which θ is minimum, as the point of concurrence of the three forces. This will give equilibrium forces which lie in the smallest possible cone of friction. (This will prevent slippage.) The components of e are then determined up to a constant factor by the condition that the three forces acting along these lines should sum to zero. Let t_i be the tangent to the gripped object at the i^{th} gripping point r_i. The point p of concurrence of the force vectors at the three gripping points satisfies $(p-r_i)^T(\mu_o n_i + t_i)=0$, $i=1,2,3$ for some appropriate constant μ_o $(=\tan\theta)$. This can be written as

$$P^T(M-\mu_o\bar{M})=0$$

where M (resp. $\bar{M}$) is the matrix whose successive rows are $(t_{ix},t_{iy},-r_i^T\bar{t}_i)$, $i=1,2,3$ (resp. $(-n_{ix}, -n_{iy}, r_i^T\bar{n}_i)$, $i=1,2,3$) and $P=(p_1,p_2,1)^T$. This is an eigenvalue problem where μ_o is the eigenvalue and P^T is the eigenvector. The real eigenvalue with the smallest absolute value should be used to determine p. Such an eigenvalue exists if, for example, the n_i's are not all in one half plane (in that case $\bar{M}$ is invertible). Once p and μ_o are determined the three equilibrium forces are simply $\varepsilon_i(n_i-\mu_o t_i)$, $i=1,2,3$, where the positive constants ε_i must be chosen to satisfy $\Sigma\,\varepsilon_i(n_i-\mu_o t_i)=0$ and $\Sigma\,\varepsilon_i=1$. These force vectors can be further multiplied by a positive factor α to guarantee a minimum gripping force magnitude.

If more than three fingers are involved in gripping the object, we can form all subsets of three fingers, use the calculation just explained to find an equilibrium vector for each such subset, and then form some suitable convex combination of these equilibrium vectors, e.g. by averaging. Notice that similar calculations hold for the case of three fingers in manipulating a three dimensional object since the concurrence point must still lie on the plane determined by the contact points. Both the matrix G and the equilibrium vector e need only be computed once during the manipulation as long as the grip points do not change.

Experimental Results

Experimental results for a *door opening* task, a *wall following* task and a *continuous rotation* are presented.

The 2-D 'door' used in our experiments is shown in Figure 6. External loads may or may not act on the door. The center of rotation is unknown to the control software. We move in the direction tangent to the circle described by the reference point (center of the knob) while keeping the forces in the normal direction to a minimum and overcoming any tangential forces (due to external loads). Treating the gripped knob as a *virtual finger* simplifies the problem to that of moving the center of the knob, while rotating the knob appropriately. The algorithm for determining the motion of the center uses the approach described earlier. We want to move in the direction tangent to the circle described by the knob and keep the forces in the normal direction to a minimum. Figures 7 and 8 give the experimental data. Figure 7 shows that during the first few steps forces build up rapidly. This is due to initial uncertainty in the estimates of the tangent directions. Figure 8 shows the corresponding behavior for the individual fingers. The force magnitude for each finger is plotted as a function of the control cycle. The system of three fingers adjusts after each cycle to maintain the grip and sustain the normal forces exerted by the door. The force behavior of each finger is different due to their different locations with respect to the door. The inner control loop adjusts the fingers to give the desired end result. The forces on each finger remain within safe limits of about 3 Newtons.

Figure 6
Door.
(Photo courtesy of Photodynamics, N.Y.)

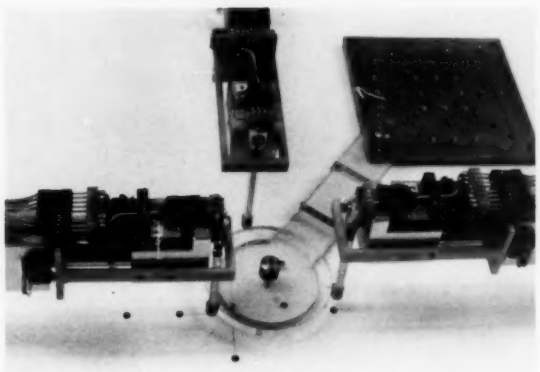


Figure 7
Forces at the Reference Point.

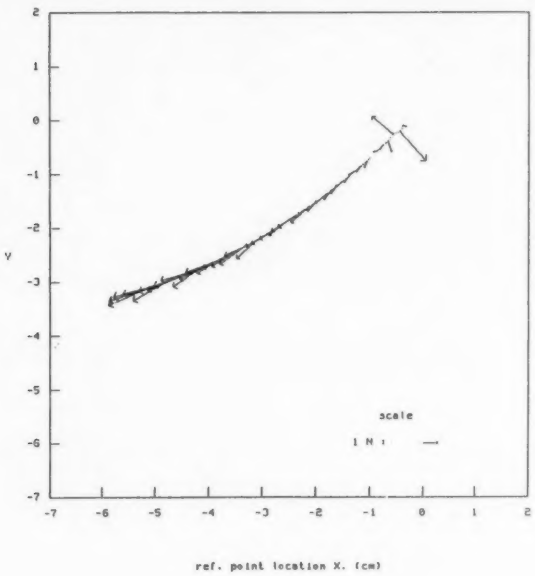
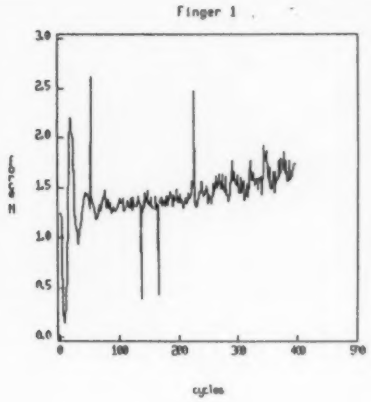
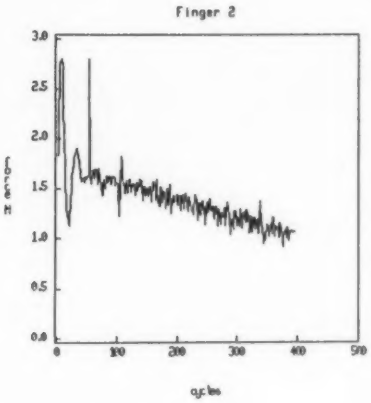
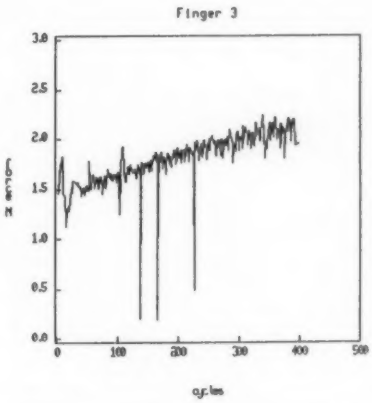


Figure 8
Finger Forces.







The configuration for the wall following experiment is shown in Figure 9. The relative position of the 'tip' of the tool (the contact point) with respect to the fingers is known but the shape of the wall is unknown. The desired contact force was 1.2 Newtons. The corresponding data is shown in Figures 10 and 11. Figure 10 shows the edge of the tracked wall with arrows representing the reaction forces on it.

Finally, Figures 12 and 13 show the experimental data for the continuous rotation task. The fingers are gripping a disk and "walk" around it repositioning themselves for the following gripping position. Figure 12 shows the path of each finger during a complete cycle of finger rotation. Figure 13 shows the shift of grasping force between one set of fingers and another during a "handover" of the disk. By concatenating very simple motions of each finger group, the "finger gaiting" routine achieves a fairly complex motion. Additional details of these and other experiments may be found in [3, 6, 9, 10].

Figure 9
Wall Following.
(Photo courtesy of Photodynamics, N.Y.)

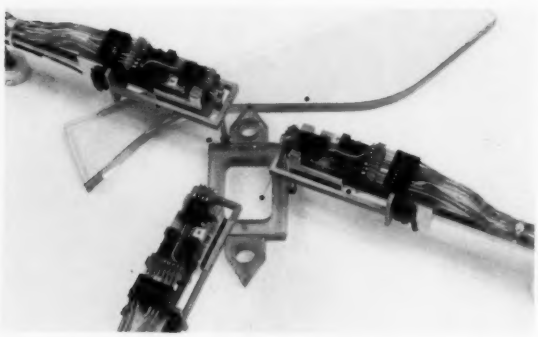


Figure 10
Contact Force.

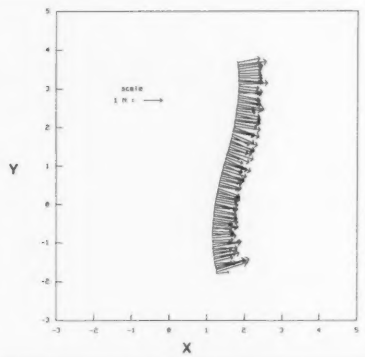


Figure 11
Finger Forces.

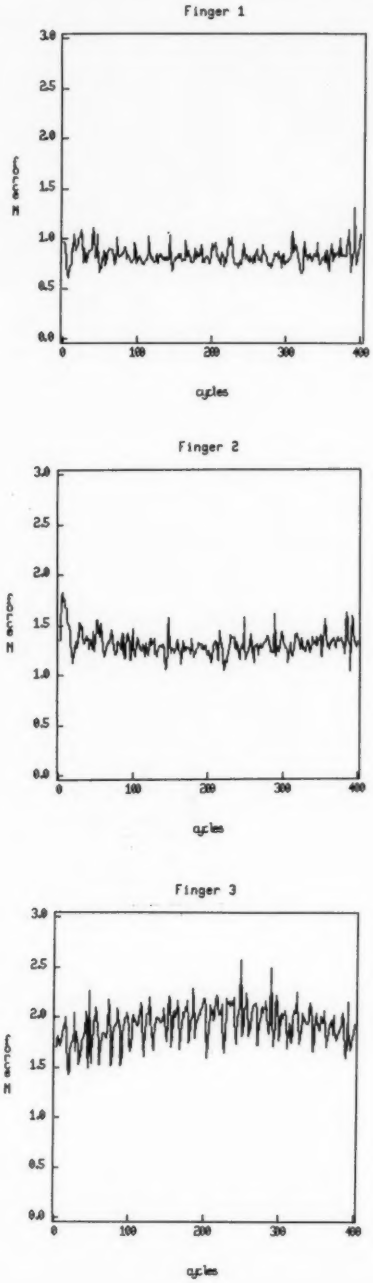


Figure 12
Finger Paths.

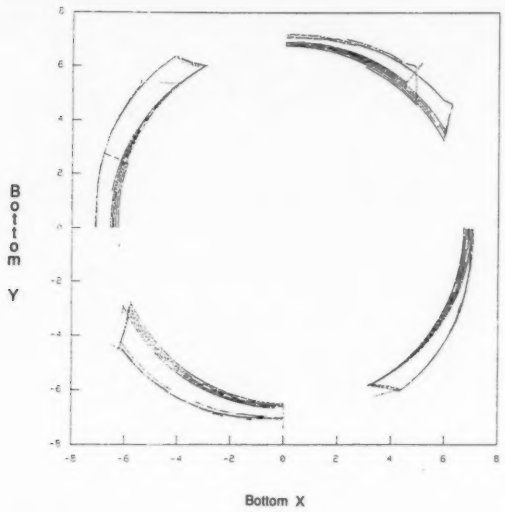
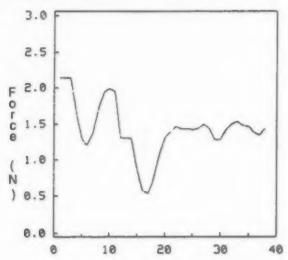
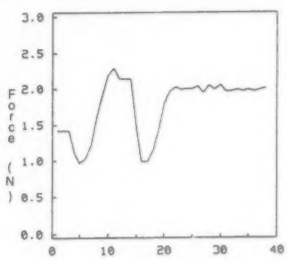
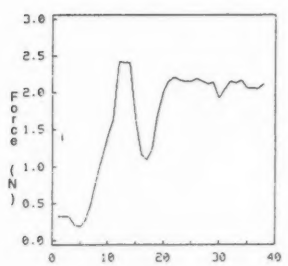
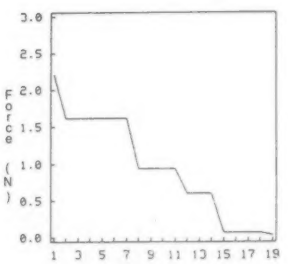


Figure 13
Finger Forces During "Hand Over".









Future Work

We will extend our research in dextrous manipulation to three dimensions by appyling the approach described above to the Utah/MIT hand. This sophisticated 3-D hand presents many new challenges but it also offers much more experimental flexibility. To guide our general research approach we have developed a list of prototypical manipulation tasks for this hand.

Most manipulation tasks involve two steps: (a) acquiring a grasp, and (b) manipulating the object once grasped. Step (a) can be broken into the following substeps:

i. locate the object

ii. choose a grasp (if the number and shape of the objects to be manipulated is limited, as in many industrial environments, this can be table driven) (see [2]).

iii. grasp the object.

We expect our work to concentrate on studies of step (b), though the prior problem of grasp acquisition is also under active study in our laboratory ([1, 12, 14]).

Here is a sample of the list of the 3-D tasks to be studied:

1. Grasp a convex object with 3 or 4 fingers, the strength of the grip being specified, and move it along a specified path.
2. Same as above, but attain continuing motion by repeatedly removing one finger from the object and repositioning it.
3. Maintain grasp on a body being moved by external forces.
4. Screw a large nut onto a bolt with a steeply pitched thread.
5. Insert a peg into a hole which fits it rather tightly.
6. Use a screwdriver to drive in an (already started) long screw.
7. Use a screwdriver to pry off the top of a paint-can lid.
8. Grip the handle of a hammer, and use it to pound in a peg.
9. Pick up a peg with a pincer and insert it into a hole.

These tasks can be classified in various helpful ways. The following are some preliminary classification parameters.

1. Can the task be performed using only the fingers, or is a wrist necessary, or is an arm necessary? Initially we plan to concentrate on tasks requiring only fingers, since this will require less infrastructure at the beginning.
2. Number of degrees of freedom that are position controlled vs. number of degrees of freedom that are force controlled.
3. What role does friction play in the problem? Is it irrelevant, as in the case of a positive grip, or must it be taken into account? (Friction may be irrelevant over the entire range of coefficients of friction (0 to infinity), or just for sufficiently small or sufficiently large coefficients).
4. Are the (force and/or position) constraints on the problem time varying (rheonomic) or time invariant (schleronomic)? If variable, do they vary slowly enough that the algorithm can treat them as constant, or are they abrupt (as in the occurrence of corners during edge following, or in catching a dropped object, or in pulling a plug)?
5. Are the environmental constraints holonomic (as with a rigidly held object) or not (for a sphere held between two planes)?

We will concentrate initially on tasks that may be performed efficiently using only force/position control derived from built in Utah/MIT hand sensors, but do intend to incorporate other sensor information later (vision, tactile).

Conclusions

We developed a control approach for multifinger manipulation. The ideas were applied to the control of a four finger planar manipulator specially built at NYU for this purpose. Results from three experiments, door opening, wall following, and continuous rotation were presented. They show promising results for the coordinated control of several fingers in a partially known environment.

References

1. C.M. Bastuscheck. *On the Stability of Grasping: Three Fingers and a Planar Polygon.* Robotics Report 98, Courant Institute, NYU, New York, January 1987.

2. Mark R. Cutkowsky and Paul K. Wright. Modeling manufacturing grips and correlations with the design of robotics hands. In *Proceedings of the International Conference on Robotics and Automation,* pages 1533-1539, IEEE, IEEE Computer Society Press, San Francisco, April 1986.

3. Christopher Fernandes and Gerardo Lafferriere. *Wall Following Experiments with the Four Finger Manipulator.* Robotics Report 128, New York University, New York, October 1987.

4. Allan Gottlieb. *An Overview of the NYU Ultracomputer Project.* Ultracomputer Note 100, Courant Institute, NYU, New York, July 1986.

5. H. Hanafusa and H. Asada. Stable prehension by a robot hand with elastic fingers. In *Proceedings of 7th International Symposium on Industrial Robots,* pages 361-368, Tokyo, 1977.

6. Maw Kae Hor. *Control and Task Planning of a Four Finger Dextrous Manipulator.* Robotics Report 124, Courant Institute, NYU, New York, October 1987.

7. Steve C. Jacobsen, E.K. Iversen, D.F. Knutti, R.T. Johnson, and Klaus B. Biggers. Design of the Utah/MIT dextrous hand. In *Proceedings of the International Conference on Robotics and Automation,* pages 1520-1532, IEEE, San Francisco, April 1986.

8. Steve C. Jacobsen, John E. Wood, D.F. Knutti, and Klaus B. Biggers. The Utah/MIT dextrous hand: work in progress. In M. Brady and R.P. Paul, editors, *First International Conference on Robotics Research,* pages 601-653, MIT Press, Cambridge, Massachusetts, 1984.

9. Gerardo Lafferriere and Deepak Mohan. *Crank Turning Experiments with the Four Finger Manipulator.* Technical Report 127, NYU Robotics Research Laboratory, New York, October 1987.

10. Gerardo Lafferriere and Deepak Mohan. *Door Opening Experiments Using the Four Finger Manipulator.* Robotics Report 100, Courant Institute, NYU, New York, March 1987.

11. Matthew T. Mason and J. Kenneth Salisbury, Jr. *Robots Hands and the Mechanics of Manipulation.* MIT Press, Cambridge, Massachusetts, 1985.

12. B. Mishra, J.T. Schwartz, and M. Sharir. *On the Existence and Synthesis of Multifinger Positive Grips.* Robotics Report 89, Courant Institute, NYU, New York, November 1986.

13. T. Okada. Computer control of multijointed finger system for precise handling. *IEEE Transactions on Systems, Man and Cybernetics,* Vol. SMC-12(3):289-299, May 1982.

14. J.T. Schwartz and M. Sharir. *Motion Planning and Related Geometric Algorithms in Robotics.* Robotics Report 79, Courant Institute, NYU, New York, August 1986.

15. Daniel E. Whitney. Historical perspective and state of the art in robot force control. *International Journal of Robotics Research,* 6(1):3-14, Spring 1987.

Biographies

James Demmel received his Ph.D. in Computer Science from the University of California at Berkeley in 1983. Since 1984 he has been on the Computer Science faculty at the Courant Institute of Mathematical Sciences, New York University. He shared the Householder Award in 1984 and the Fox Prize in 1986, both in numerical analysis. He is a Presidential Young Investigator.

Gerardo Lafferriere is an Associate Research Scientist of the Robotics Research Labortory at the Courant Institute of Mathematical Sciences, New York University. He received his Ph.D. in Mathematics from Rutgers University in 1986. His research interests are in the areas of nonlinear control theory and robotics. His most recent work is on the control of dextrous robot hands.

Jacob Schwartz is Professor of Mathematics and Computer Science at New York University and Director of the Computer Science Division of the Courant Institute in New York. He has served on several editorial boards on applied mathematics and computer science. Besides being a member of the National Academy of Sciences, Jacob Schwartz was the co-recipient of the Steele Prize of the American Mathematical Society. He is currently acting as the Director of ISTO at the Defense Advanced Research Projects Agency in Washington, DC.

Micha Sharir is a Visiting Research Professor of Computer Science and Associate Director of the Robotics Research Laboratory at the Courant Institute of Mathematical Sciences, New York University, and a Professor of Computer Science at Tel Aviv University. His publications span the areas of motion planning, computer vision, and other theoretical problems related to robotics, as well as computational geometry.

Molecular Biology of Marine Microorganisms: Biotechnological Approaches to Naval Problems

by Thoyd Melton, Office of Naval Research and John W. Bodnar, Northeastern University

Introduction

"Over the centuries, man has become increasingly adept at harnessing the biologic world . . ."– David Baltimore

The Biological Sciences Division at ONR supports basic research in molecular biology which focuses on biochemistry, marine microbiology, biopolymers, biosurfaces, extreme environmental habitats, and molecular recognition. The programmatic and scientific objectives of the Molecular Biology Program are directed towards the development of molecular tools to investigate complex marine microbial communities; gaining an understanding of the molecular biology of marine microorganisms; and providing a knowledge base necessary for the biotechnological exploitation of these organisms. Research supported by this program provides insights into molecular biological processes, relationship of three dimensional structure of biomolecules to their physical properties and molecular responses of marine organisms to the marine environment. The Molecular Biology Program addresses basic research issues pertinent to specific naval needs: control of biofouling and biocorrosion; biosensors for chemical and biological detector systems; and novel materials and catalysts based on biopolymers. These naval needs drive the basic research currently supported in the Molecular Biology Program at ONR. The purpose of this review is to highlight research contributions of ONR contractors in the Molecular Biology Program. Specific research areas reviewed are biofouling and biocorrosion; novel materials; microbes of extreme environmental habitats and molecular symbiosis.

Biofouling and Biocorrosion

Growth of marine organisms on ship hulls and underwater surfaces is costly to the Navy in terms of operating efficiency, readiness and dollars. The layer of organisms that covers the underwater surfaces on a ship can reduce its top speed, increase fuel consumption, reduce the efficiency of heat exchangers and condensers, and even induce corrosion. In the Navy shore-establishment about $800 million is spent each year to maintain structures including approximately $150 million just in coating operations (36). Antifouling coatings have been developed to prevent or reduce biofouling, but these have been only moderately usually being toxic and hazardous to workers applying or removing them.

The Office of Naval Research has supported basic and applied research to understand the mechanisms of biofouling and biocorrosion. Recent advances in molecular biology, genetic engineering and biotechnology have opened up new research horizons and approaches to these problems. If we can understand the biochemistry, genetics, and physiology of the organisms that foul underwater surfaces and the mechanisms that underlie their ability to attach to surfaces and cause corrosion, then it may be possible to inhibit specific steps in the formation of the fouling layer or inhibit the biochemical reactions that induce corrosion.

Biofouling and biocorrosion proceed through several steps (56). First, free-living microorganisms in the water column must sense the presence of a surface either by physical or chemical cues. After their initial recognition a series of complex events follow which allows the organism to attach to the surface. Corrosion may result from biochemical reactions in the metabolic processes of the attached organisms.

Recognition of Surfaces by Microorganisms

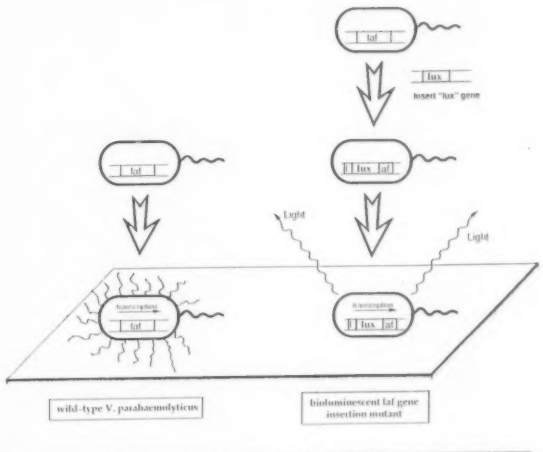
Recognition of an underwater surface involves complex biochemical changes within an organism in response to the surface itself or to chemicals released from the surface by organic films or other organisms. Experiments with the bacterium *Vibrio parahaemolyticus* and with the Pacific red abalone *Haliotis refescens* have provided insights into the molecular mechanisms of these changes.

The bacterium *V. parahaemolyticus* produces a single flagellum which propels it through the water allowing it to be free-swimming. When it contacts a solid surface (or is grown in the laboratory in viscous medium), the cells elongates to about twenty times their original length and each cell produces hundreds of lateral flagella which allow it to "swarm" over the surface. If a cell is then removed from the surface (or the viscous medium), the elongated cell divides into shorter cells and the lateral flagella are lost.

The complex behavioral responses appear to be mediated by changes in gene expression of *V. parahaemolyticus* that are induced by the viscosity of the medium (4). Dr. Michael Silverman and colleagues at the Agouron Institute, La Jolla CA, have identified the gene which controls the formation of the lateral flagella (*laf* gene) using a technique called insertional mutagenesis. Through the use of a DNA vector that contains genes for bioluminescnce (*lux* genes) these bacteria can be transformed to produce proteins which will emit light (20). As shown in Figure 1 the DNA vector introduced into *V. parahaemolyticus* is randomly integrated into the DNA of the bacterial genome. If a particular vector DNA is inserted, by chance, into *laf* gene, the inserted DNA could have two effects: 1) it could change the coding sequences of that gene and inactivate it or 2) it could (if inserted in the proper orientation) be under control of the DNA sequences which regulate the *laf* gene, thereby leading to expression of the *lux* gene at the expense of *laf* gene expression. These mutant bacteria would not be able to produce the *laf* gene products and could not swarm, but when conditions were right for swarming (i.e. they were grown in viscous medium) the *lux* genes would be activated instead. These mutants could be identified easily since they would not swarm but would glow when grown in viscous medium. After growth of many individual bacterial colonies in viscous medium several colonies with these characteristics were isolated.

Figure 1

Genetic engineering of the lateral flagella (laf) genes of V. parahaemolyticus via insertional mutagenesis. Mutants constructed by this technique contain the genes for bioluminescence (lux) inserted in the laf gene. Expression of the lux genes is controlled by the laf regulatory elements.



The genetically engineered bioluminescent *V. parahaemolyticus* showed not only the complex morphological changes depended on activation of a single gene but provided a powerful means by which the swarming response could be studied. When the mutants are grown under conditions that would normally induce swarming in the wild type parent bacteria, they will glow instead. Therefore, it was now possible to study regulation of the "swarming" response, which is essential to the bacteria ability to attach to surfaces, using bioluminescence as a marker (4). A wide variety of chemical agents including cations, anions, biological inducers, and organic chemicals were examined to see if they could induce light. No agent was identified which lead to bioluminescence, and consequently induced the attachment response.

Only changes in viscosity of the medium induced the bacteria to glow, and only the viscosity, not any particular gelling agent, elicited the response. This suggested that the bacteria are able to sense the physical characteristics of their environment and that the morphological changes in the cells are the result of genes expressed induced by the physical changes in their growth environment.

Chemical signals that can be used by fouling organism to identify a suitable attachment surface have been studied by Dr. Daniel Morse of the University of California, Santa Barbara (3). Larvae of the Pacific red abalone, *Haliotis rufescens* are free swimming, but must settle on a substrate to allow them to develop into adults. This settling is induced by peptide(s) found on the surfaces of certain red algae, species in the absence of the inducing peptide the larvae fail to settle, and consequently develop new metamorphose into adults. While the nature of this peptide is still unknown its mode of action is similar to the amino acid neurotransmitter gama-aminobutyric acid (GABA), and in the laboratory GABA can be to induce the larval setting.

Biochemical assays that measure the response of the larvae to GABA or synthetic GABA analogs indicate that the induction of larval settling follows a pathway that is similar to the biochemical pathways for transmission of signals through the nervous systems of both vertebrates and invertebrates. The peptide or GABA binds to a cell surface receptor protein and this binding induces changes across the cell membrane resulting in cyclic AMP formation within the cell. The cyclic AMP then acts as a second messenger to cause multiple changes within the cell one of which allows the chemical signal to be passed to adjacent cells in the nervous system. This response can be modified by another pathway which is mediated by a diacylglycerol second messenger, this second message is regulated by a G binding protein at the cell membrane. This second pathway is induced by soluble diamino acids. The abalone larvae sense the presence of the proper algae through the GABA-like peptide and sense the presence of an environment rich in nutrients through the diamino acids. These chemical signals send a message through the nervous system to trigger the complex biochemical changes required for the development that cue into an adult.

Once the physical and chemical signals of surface recognition are known and their biochemical pathways analyzed, this knowledge can be exploited to prevent or greatly reduce fouling. For example, an analog of the GABA-like peptide of the abalone could be synthesized which binds to the receptor but does not allow further signal transmission, and thus, not provide a positive signal for confusing the organism's sensor system. If this sort of analog could be incorporated in the surface of a hull coating, it could yield a nontoxic coating which prevents attachment of organisms.

The means by which diatoms sense a surface, and subsequently adhere to it is being invested by Dr. Keith Cooksey at Montana State University. This study explores the chemical specificity of the sensing process and its diversity in fouling organisms of the geneus Amphora. Dr. Cooksey's research also focuses on the identification of membrane receptors which bind sensed compounds and thus function as initial sensory transducers.

Attachment of Biofouling Organisms to Surfaces

After organisms identify an appropriate surface for settlement they will, in most cases, change in some manner to allow attachment to that surface. This process usually includes the production of an adhesive compound and/or the production of specialized structures. Adhesion of bacteria to underwater surfaces can be the first step in the coating of surfaces leading to the establishment of a biofilm. This microbial film can be used, in turn, as an attachment site for other microorganisms followed by a variety of inverterbrates (56).

The coating substances and adhesives are under investigation in several ONR supported laboratories. Collectively the data suggest that, in general, these substances are composed of polysaccharide or polypeptide polymers usually associated with other proteinous components. The "slime" produced by the gliding associated (Cytophagales) appear to be comprised of a homopolysaccharide of galactosamine in association with at least eight proteins (R.P. Burchard, personal communication). The holdfast of the Caulobacters, which anchors the bacterial cells to a surface is composed in part by polymers of N-acetyl-glucosamine and contains one major protein whose gene has been isolated (J. Smit, personal communication). In contrast, the adhesive for the marine mussel consists primarily of a polypeptide polymer (55).

Future research may allow for the biochemical identification of component(s) that allow binding of organisms on surfaces. With this knowledge it should be possible to develop synthetic analog polymers that would prevent the attachment of organisms and such polymers could be used in coatings to prevent or reduce fouling on surfaces exposed to the marine environment.

Mechanisms of Biocorrosion

Organisms within the biofouling community can be a cause of corrosion which can damage hulls and underwater fittings. The major source of the biocorrosion is the archaebacteria which can utilize exotic materials in their metabolism. Some types of archaebacteria which may be involved include methanogens (methane producers), sulfate reducers, sulfur oxidizers, nitrate reducers, manganese oxidizers, and thermophiles (bacteria which can grow at high temperatures). This involvement of archaebacteria in marine corrosion has been reviewed earlier in *Naval Research Reviews* (56).

Recently it has been shown by Dr. Lacy Daniels at the University of Iowa that one class of methanogens can actually use mild steel as an energy source for growth (13). These bacteria produce methane which is apparently synthesized from hydrogen and carbon dioxide during growth. However, several strains of methanogens can use carbon dioxide and elemental iron. These strains can be successfully grown in media which contain only nitrogen, dioxide, and mild steel. Clearly understanding the unusual pathways of metabolism in the archaebacteria will be helpful in understanding and preventing corrosion of underwater components.

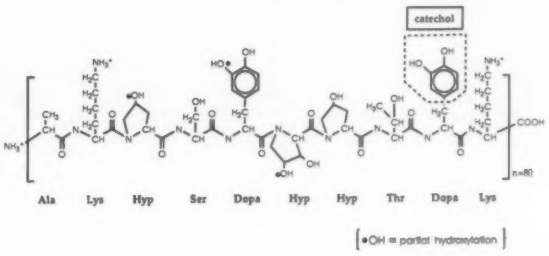
Novel Materials

One of the most exciting directions that research on the molecular biology of marine organisms might lead is in the development of biotechnology to manufacture novel materials. Biological materials have the advantage that they can be nontoxic, biodegradable, not dependent on petroleum for their production, and extremely specific in their mode of action or function. Some of the current biotechnology research supported by ONR has potential in the development of genetically engineered underwater adhesives, microcapsule coating films, magnetic materials, biodegradable plastics, and genetic engineered organisms suitable for use in bioreactors.

The marine mussel attaches itself to solid surfaces by an adhesive which consists of a long protein polymer. This polymer is composed of about eighty copies of a repeating sequence of amino acids shown in Figure 2; these are modified in the organism in specific ways for adhesion to surfaces, spreading and curing (55). The phenylalanine side chains in the protein are modified to catechols which have a very high affinity for metals or metal oxides; this modification probably serves to increase the binding of the adhesive to the substrate. The numerous proline and hydroxyproline residues tend to destabilize conformation of the polypeptides; probably ensuring that the adhesive forms a foam that spreads over surfaces hastily. Lastly, because curing is essential to the development of cohesive strength, cross-links are required between polymer chains. The marine mussel adhesive could have two types of cross links, one to soluble iron or A1+3 cross-linked to two or more catechol side chains; while in a second case covalent bonding of lysine amines on one chain are linked to catechols on another. Therefore, the natural polymer adhesive has several distinctive features which would be useful in synthetic adhesives.

Figure 2

Repeating peptide of the mussell adhesive protein.



Dr. J. Herbert Waite of the University of Delaware in collaboration with the GENEX Corporation has begun to bioengineer such a synthetic adhesive. A DNA sequence which encodes the repeating amino acid sequence in the mussel adhesive has been synthesized, introduced into both bacteria and yeast, and a totally synthetic adhesive polymer analog has been produced in these genetically engineered organisms. It is hoped that this polymer will be a prototype for a synthetic underwater adhesive or a metal primer.

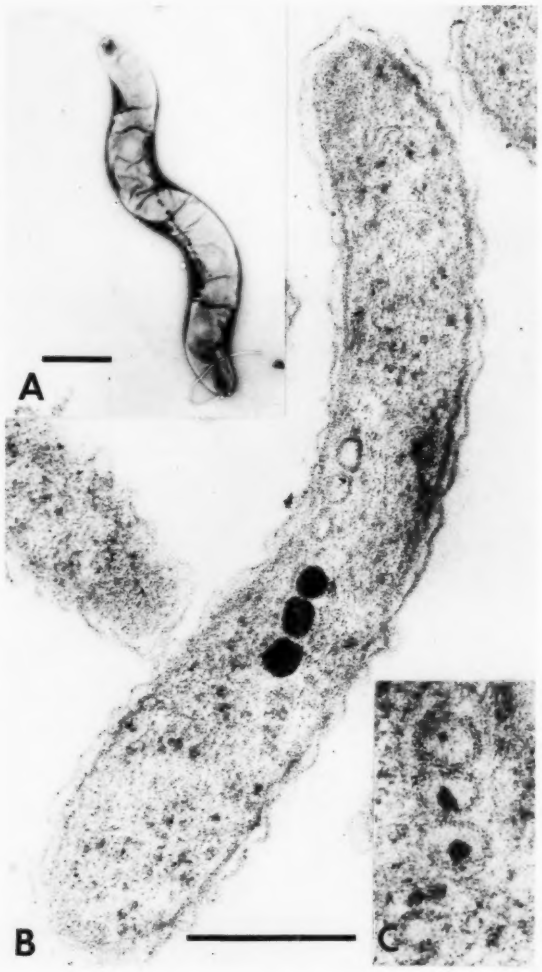
A similar protein polymer that also contains catechol in modified side chains is used by the liver fluke to coat its egg capsules preventing their degradation in the digestive tract. This repeating polypeptide has been characterized biochemically and is being genetically engineered as a potential coating for synthetic microcapsules (A. Ficht, personal communication). The unusual properties of the catechol side chain may also serve as a basis for synthetic polymers suitable for semiconductors, electrocatalysts, batteries, or molecular information systems.

A group of motile bacteria has been found which responds to the earth's magnetic field. This adaptation is believed to allow these bacteria to find the sediments of diverse aquatic habitats (and potentially a better food supply) by swimming along lines of magnetic flux which are inclined in most latitudes (5). This hypothesis is strengthened by the fact that Northern hemisphere magnetic bacteria swim toward the opposite magnetic pole than Southern hemisphere bacteria although both swim toward the sediments of their normal habitat. These bacteria sense the magnetic field through the presence of magnetosomes, which are cellular inclusions that contain magnetic iron crystals. The magnetosomes are aligned within the organism (see Figure 3) so that a single bacterium is in essence a tiny biological magnet. Methods have been developed in the laboratory of Dr. Richard Blakemore at the University of New Hampshire to isolate pure magnetosomes, and work is in progress to identify and engineer the genes which control their biosynthesis (Blakemore, personal communication). Biologically produced microscopically sized magnets have potential for use in electronics and computer technology or in catalytic devices (35).

Inclusions produced in the cells of another bacterial group may be the basis for biodegradable plastics. When grown in media containing n-alkanoic acids, bacteria of the genus *Pseudomonas* will synthesize intracellular granules which are composed of the polymer poly betahydroxyalkanoate or PHA. These granules can comprise up to thirty percent of cellular weight, and can serve as an energy source when they are starved for carbon substitutes. Drs. Robert Lenz and R. Clinton Fuller of the University of Massachusetts at Amherst have shown that these granules can be extracted and can be the raw material for plastics. Experiments are in progress to optimize the production of PHA during growth and to optimize the mixture of alkanoic acids in the medium which allow synthesis of the strongest PHA polymers. Since PHA was synthesized by the pseudomonads as a carbon source for growth any plastics based on these polymers should be readily degraded by these bacteria. Therefore, these bioplastics should find use in containers which will maintain their strength during normal use but which can be quickly degraded to carbon dioxide and water when dumped at sea or in landfills and exposed to environmental pseudomonads.

Figure 3

Transmission electron micrographs of Aquaspirillum magnetotacticum. Cells contain intracellular crystals of the mixed valence iron oxide magnetite. (A) Intact cell negatively stained with uranyl acetate showing a string of magnetosomes arranged along the axis of cell motility. Bar = 1 μm. (B) A portion of a thin-sectioned cell which has been starved of iron prior to preparation for electron microscopy. In addition to magnetosomes present, membranous vesicles devoid of iron but of the appropriate dimensions and arrangement of magnetosomes are evident within the cell. Bar = 250nm. (C) A highly magnified image of magnetosome boundary membranes similar to those in (B) but containing yet uncrystallized amorphous material rich in iron. Electron micrographs provided by Dr. Richard Blakemore, Department of Microbiology, University of New Hampshire.



Microbes of Extreme Environmental Habitats the Archaebacteria

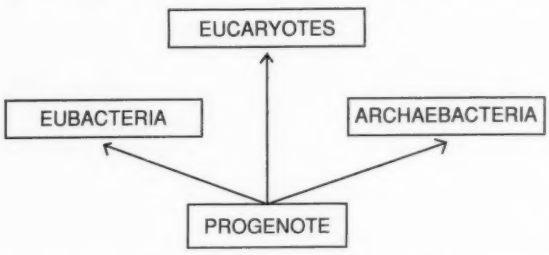
The study of microorganisms that thrive in extreme environmental habitats offers an opportunity to understand molecular mechanisms evolved for adaptation to such habitats, identify natural substances stable in extreme environments and reveal principles applicable to macromolecular design. Research supported by the Molecular Biology Program at the Office of Naval Research (ONR) in this area responds to the Navy's need for stable biomolecules and the biotechnological tools to produce them. The major group of organisms adapted to thrive in extreme environments consists of the recently discovered archaebacteria.

The archaebacteria form a third kingdom of organisms evolutionarily distinct from the eukaryotes and eubacteria (see Figure 4). They represent a gene pool of diversity and evolutionary adaptions of which little is known. Three kinds of bacteria make up this unusual kingdom of organisms: bacteria that flourish in hot acidic sulfur springs, those that tolerate and require high salt concentrations to live and the third group, the methanogenes, includes bacteria capable of producing methane which live in environments devoid of oxygen.

The archaebacteria were first proposed as a distinct taxonomic unit by Dr. Carl Woese and his colleagues at the University of Illinois (58,59,60). The grouping of these bacteria was initially assigned on the basis of partial (rRNA) sequence comparisons. More recently, comparisons of complete 16S rRNA sequences have been used to refine archaebacterial phylogeny. From these investigations it is clear that the archaebacteria constitute a phylogenetically coherent group of organisms that excludes the eubacteria and the eukaryotes.

Figure 4

The lines of evolutionary descent which provide the basis for the modern-day three primary kingdoms.



Molecular Biology of Archaebacteria

Research supported by ONR represents a multidisciplinary approach to the study of the molecular biology and functional environmental role of the archaebacteria. A primary objective of this research is to determine the mechanisms that underlie the ability of these bacteria to exist in extreme environments (boiling acid, high salt, and high temperature) which would damage other cells. The focus of the ONR program is on molecular basis for salt, acid, and thermal stability of cellular components; the biochemical and physiological mechanisms by which the archaebacteria have adapted to extreme environments; the identification of unusual biochemical transformations characteristic of the archaebacteria; the development of molecular genetic tools to exploit these organisms biotechnologically and the genetic diversity and ecological role of their organisms. It is essential that archaebacterial molecular biology be developed in order to exploit the unique biochemistry and physiology of these unusual microorganisms.

Recently, Dr. Woese has shown that archaebacterial phylogeny is not one of three distinct branches corresponding to the three archaebacterial phenotypes i.e., halophiles, methanogens, and thermoacidphiles. Rather it appears that the thermophilic sulfur-dependent archaebacterial phenotype is intermixed with the other two phenotypes. The extreme halophiles have been shown to branch within the methanogens (62). Dr. Woese has also shown that the thermoacidophilic archaebacterium, *Thermoplasma acidophilum,* is more closely related to the methanogens and halophiles than to the other thermophilic archaebacteria. These results suggest that the thermophilic archaebacteria may represent the basic ancestrial archaebacterial phenotype. Dr. Woese postilates that all life may, to some extent have a thermophilic origin. This would indicate that perhaps the other two bacterial kingdoms might have arisen from within the archaebacteria, making it the "urikingdom" from which other kingdoms evolved.

Organization and Structure of Archaebacterial Genes

Many advances have been made in the study of the molecular biology of the archaebacteria. This subject has been the focus of two recent reviews published by ONR contractors (14,27). Some of the molecular features of archaebacterial RNA genes which have been elucidated includes the organization, transcription, and processing of stable archaebacterial RNA genes. The rRNA genes have been cloned and sequenced and the sequence data used to establish phylogenetic relationships and determine gene organization and structure (15,60). The RNA genes in archaebacteria appear to exist in one to four copies per genome and have the order 5′-16S-23S-5S-3′. In some cases (i.e. several halopilic and methanogenic species) one can find an alanyl-tRNA gene present within the 16S-23S intergenic space. Even though

the 16S and 23S rRNA genes in the sulfur-metabolizing archaebacteria are linked, alanyl-tRNA genes do not appear in the intergenic regions.

Sequence data of more than 40 separate tRNAs from *Halobacterium volcanii* indicate that these genes do not encode the CCA-3′ termini of mature tRNAs (23,24). The tRNA genes are not only found associated with rRNA genes but may exist singly or in clusters. The serine, tryptophan, and leucine tRNA genes have intervening sequences (15,57). Both presence of intervening sequences and the lack of the 3′terminal CCA residues in the tRNA gene are characteristics of *H. volcanii* and are found in common with eucaryotic tRNAs.

In addition to the progress which has been made regarding the elucidation of the organization and structure of RNA genes in the archaebacteria, the organization and expression of genomic DNA in the archabecteria is documented. The DNA from a number of methane producing bacteria has been cloned into *Escherichion coli*. It has been possible to rescue *purE, hisA, proC and argG E. coli* auxotrophs with the cloned fragments from the methanogenic bacteria (11,28). A high degree of complementation occurs in genomic DNA between the eubacteria and archaebacteria. These bacterial groups share some homology with respect to promoter consensus sequence. Transcriptional regulation has been demonstrated to occur for at least one amino acid gene of *Methanococcus voltae*. A two fold increase in the mRNA for this gene is observed in the presence of inducers (5).

Gene Transfer Systems

The advancement of archaebacterial molecular biology is greatly dependent on the development of gene transfer systems in these organisms. The development of such systems affords the opportunity to introduce and propagate recombinant DNA molecules in the archaebacteria. ONR contractors are important contributors to the development of archaebacterial gene transfer systems. Recently, a transformation system for the archaebacterium *Halobacterium volcanii* has been reported (7). A plasmid has been characterized which is present in about six copies per chromosome. The characterization of this plasmid makes possible the construction of shuttle vectors of plasmids for *H. volcanii* and other archaebacteria.

Sequence data on the isolated plasmid reveals the presence of several open reading frames of which four may encode polypeptides of 189, 228, 200 and 808 amino acids. Codon usage is similar among these ORFs. It has been possible to generate a plasmidless strain of *H. volcanii*, which can be restored through transformation.

Dr. Doolittle and his colleagues have been instrumental in the development of a transfection system (i.e., transformation DNA) in *H. halobium* with bacteriophage øH DNA (9). The linear phage DNA molecular produced between $5-10^6$ and $2-10^7$ transfectants per ug DNA. Survival and regeneration of *H.halobium* spheroplasts were very efficient. A natural transformation system has also been discovered in *H. vocanii* (37).

Evidence has also been presented for a natural transformation system in the thermophilic methanogen *Methanobacterium thermoautotrophicum* Marburg (6). The wild-type strain of this methanogenic archaebacterium was transformed by high-molecular weight DNA. These recent developments in gene transfer systems greatly contribute to the molecular genetic tools needed to explore genomic organization and structure in the archaebacteria.

DNA Insertion Elements

The development of genetics in novel strains has been greatly aided with the discovery of naturally occurring endogenous DNA insertion (IS) elements. DNA insertion elements are transposable. IS elements vary in length from a few hundred to a few thousand base pairs. A common feature of all IS elements is the presence of inverted-repeat sequences at the termini. In nature IS elements promote gene amplification and deletions and provide a mechanism for reassorting gene segments and control expression of certain genes. IS elements have also proven to be effective molecular genetic tools.

The discovery of endogenous IS elements in the archaebacteria offers an opportunity to isolate novel mutants, manipulate gene expression, enhance gene and strain constructions, and allow for programmed gene rearrangements in these organisms. Spontaneous genomic rearrangements (i.e., transpositions, deletions, or recombinations) have been associated with the presence of repeated DNA sequences in *H. halobium* (48). It has been estimated that the genome of *H. halobium* contains as many as 50 copies of repeated DNA sequences (47). A large degenerate family of insertion sequence-like elements have been identified in the genome of the archaebacterium, *H. volcanii* (26). This family of IS is present 20-30 copies per genome but the elements have not been shown to be mobile in *H. volcanii*. Presently, it appears that many members of these insertion sequence-like elements are not transposable. A heterogenous family of different insertion elements have been found to exist among the genomic DNA of *H. halobium* that are also found on plasmid DNA (44).

Stability of Archaebacterial Proteins and Protein-DNA Complexes

Extremely halophilic bacteria live in salt concentrations that exceed the salinity of seawater by 30-50×. ONR continues to support basic research directed towards an understanding of the molecular basis of halophiles protein stability and function.

A common feature of macromolecules in halophiles is an abundance of acidic amino acid residues and a deficit of basic residues. Compared to proteins from non-halophales they appear to have a large amount of bound salt and water. These features may contribute to the structural stabilization of these proteins at high salt concentrations.

The molecular properties of nucleic acid-protein interactions at high salt concentrations in the halophilic bacteria is also under investigation by ONR contractor, Dr. Henryk Eisenberg. Of particular interest is the archaebacterial RNA polymerase and its interaction with DNA promoter regions. *Halobacterium marismorui*, Dr. Eisenberg is using the binding of RNA polymerase to promoters as a model for studying the interactions of DNA with protein. Attempts are being made to adapt DNA foot-printing-analysis to high salt conditions as a means to study protein-nucleic acid complexes under these conditions. This novel approach will provide a method to determine the nucleotide sequence of the polymerase binding site in the promoter region under high salt conditions. Other halophilic proteins subjected to structural studies include glutamate dehydrogenase, malate dehydrogenase, ferredoxin, ribosomes and flagellins (19).

Archaebacteria Membranes and Lipids

Since the archaebacteria exist in extreme environmental habitats, they are a promising source of novel metabolically active compounds. It is thought that such compounds may contribute to the adaptability of the archaebacteria to extreme habitats. Much research has focused on the unique membrane properties of the archaebacteria. Our concept of the structure of biological membranes comes primarily from research done on eukaryotes and the eubacteria. The paradigmatic membrane consist of polar lipids with two unbranched hydrocarbon chains arranged in a bilayer containing some membrane proteins in a fluid mosaic arrangement. Because the lipid composition of the archaebacterial membrane are quite different, the model is inappropriate for the archaebacteria. These differences in lipid composition are illustrated in Table 1. Archaebacteria membrane lipids are based on a branched C-20 hydrocarbon chain and are composed of either diphytanylglycerol diether or diphytanyldiglycerol tetraether. Another unique feature of archaebacterial lipids that likely contributes to their stability is the presence of ether linkages between the glycerol backbone and the hydrophobic chains. Unlike the ester linkages found in eubacteria and eucaryotes, these bonds are more stable to acid and thermal hydrolysis. The glycerol lipid C-2 configuration is L in the archaebacteria instead of R as found in the eukaryotes or eubacteria. The lipids of archaebacteria lack unsaturation (i.e., labile C=C, N=S on C=O bonds). There is evidence that the classic bilayer membrane structure is altered in the archaebacteria such that the membrane may be a monolayer or a mixture of mono- and bilayer structures.

TABLE 1

Features of Archaebacterial, Eubacterial and Eukayotic Membranes

Membrane Components	Archaebacterial	Eubacteria	Eucaryotes
major membrane lipid	byphytanyl, and/or phytanyl	fatty acid	fatty acid
major membrane lipid to gylcerol linkage	ether	ester	ester
glycerol lipid C-2 configuration	L	R	R

Given the unusual properties of the archaebacterial lipids, it is possible they may process novel properties. Dr. E. Chang of the Naval Research Laboratory (NRL) has initiated research to study the physical properties of the lipids of thermoacidophilic archaebacteria. Glycerol dialkyl-glycerol tetraether (GDGT) hydrolyzed lipids have been purified. The GDGT has been used to form stable liposomes. These liposomes are presently being characterized using fluorescence and light-scattering techniques. A very interesting spin-off of this project is the observation that the archaebacterial lipids may function as thermally and chemically stable boundary lubricants.

Extremely Thermophilic Archaebacteria

Recently much attention has been given to the study of microorganisms living in extreme environmental habitats. A recent major scientific discovery is the existence of complex ecoysystems at hydrothermal vents or deep-sea hot springs found at sea floor spreading centers (52). Hydrothermal vents vary from low temperature (25 °C) fluid discharges, to high temperature (350 °C) black smokers. In 1977, geologists on the Navy research submarine Alvin discovered animal life previously unknown to science at 2600 meters below the ocean surface. Photoautotrophs are usually the primary producers in most relatively shallow marine and terrestrial ecosystems, however, in the permanently dark hydrothermal vent environments deep sea chemolithotrophic bacteria are the primary producers (33,29).

The interaction of these bacteria with the animals found at the hydrothermal vents will be discussed in more detail in the section of this review dealing with marine symbiosis. ONR supports research to investigate the complex ecosystems found at the deep sea hydrothermal vents since microorganisms found there could be a source of novel materials and of biotechnological importance.

With the support of ONR, Dr. Holger Jannasch of Woods Hole Oceanographic Institute has studied extremely thermophilic archaebacteria isolated from the geothermally heated sediments at the Guaymas Basin Hydrothermal vent in the Gulf of California. Unlike the vents of the Eastern Pacific Ocean, the Guaymas Basin hydrothermal vent site is overlayed by sediments of terrigenous origin (34,50). The sediments are a rich composite of diatoms, detrital clay, calcareous nanofossils, feldspar, quartz and organic carbon. Hydrothermal fluid emmissions at the Guaymas Basin vent site reach the ocean floor at a depth of 2000 m with exit temperature of 150-355 °C.

Dr. Jannasch and his colleagues have been studying hydrocarbons which are abundant in these sediments. They have an unusually high degradation of alkanes at low temperatures. A large number of extremely thermophilic isolates have been obtained from this site, which are archaebacterial fermenters classified in the genera *Desulfurococcus* and the *Thermococcus*. Their optimal growth temperatures range from 85 ° to 94 °C. Efforts to isolate an effective alkane degrading bacterium from sediments of Guaymas Basin yield mostly aerobic, mesophilic eubacterial strains. No viable microorganisms could be isolated in black smoker hydrothermal fluid in the absence of ambient seawater. Those bacteria isolated were incapable of growth when incubated under pressure (1-250 atm] and high temperatures (100-250 °C). Some researchers have reported a consortium of bacteria to grow temperatures of at least 250 °C (1,2) and have more recently obtained evidence of methane oxidation by these bacteria.

Molecular Marine Symbiosis

Symbiosis in the Marine Environment

The marine environment is a rich complex ecosystem consisting of consortia of microorganisms and animal life. An enormous variety of symbiotic relationships exit in the marine environment and range from loose consortia to obligate associations. Table 2 list some of the more familiar marine symbiotic relationships. A fundamental understanding of marine microorganisms and their interactions with other marine organisms is vital to our understanding of the marine environment as a whole. ONR has recently targeted support for research in the area of molecular marine symbiosis. Given the recent advances in genetics, molecular tools (i.e., powerful genetic systems, directed gene manipulation, and protein engineering) now exist to investigate complex marine communities.

Marine symbiotic systems are important to study since they: 1) can be considered as natural bioreactors in which host and symbiont physiologically adjust to each other so as to optimize bioenergetic and metabolic processess during the association; 2) offer opportunities for novel genetic regulation and engineering; 3) provide an alternative to more familiar modes of evolution; and 4) provide systems which possess novel mechanisms or processes which are absent from either partner when not in symbiotic association. Some of the key issues concerning marine symbiosis identify specific host and/or symbiont factors required for the establishment and maintenance of symbiosis; and identify the vibruent and energetic exchanges essential for a successful symbiosis.

In order to study symbiosis in the marine environment, an appreciation of the complexity of natural marine microbial communities is required. These complex communities may exist in marine sediments, hydrothermal vents, biofouling films on marine surfaces and marine snow. ONR supports efforts to provide novel molecular tools to investigate the molecular interactions of symtiotic organisms. The single most important issue impeding the identification, enumeration, and consequently the genetic manipulation of symbiotic marine microorganisms, is the inability to culture most of the microorganisms found in the marine environment. Progress in the study of marine symbiotic and free-living microorganisms greatly depends on the development of novel techniques to enumerate natural marine populations.

Table 2

Examples of Marine Symbiotic Systems

Host	Symbiont
Hydrothermal Vent Invertebrates	Chemoautotrophic Bacteria
Marine Invertebrates	Photosynthetic Bacteria Chemoautotrophic Bacteria Heterotrophic Bacteria
Fishes	Bioluminescent Bacteria
Amoeba	Micro Algae
Prochordates	Micro Algae

ONR contractor Dr. Kenneth Nealson of the University of Wisconsin, Milwaukee is developing molecular tools to study natural marine populations. He and his colleagues are applying recently developed molecular techniques to create hydridization probes to study the distribution and community structure of non-culturable free-living and symbiotic marine bioluminescent bacteria. Most of these symbiotic bioluminescent bacteria have not been previously identified or cultured. The most exciting aspect of this work is the potential use of the techniques developed to study any group of bacteria.

Dr. Nealson is developing two types of probes: 1) function-specific oligonucleotide probes for the bioluminescent *luxA* gene (i.e., the alpha subunit of the enzyme luciferase, and 2) 16S ribosomal RNA probes for general group analysis. Unlike many of the standard methods presently used for the enumeration and estimation of bacteria these techniques do not depend on the primary isolation and pure culturing of the bacteria. These molecular techniques are far more sensitive, specific, rapid and easier to use than procedures presently in use. Figure 5 outlines the hybridization approach to probing natural populations. The construction and the application of the *luxA* gene as a function-specific probes permits the identification of different species of bioluminescent bacteria in natural marine populations. Dr. Nealson presently is sequencing and analyzing the 16S rRNA from a number of luminous bacteria in order to develop probes for direct identification of the genus of individual bioluminescent bacteria present in natural samples.

The 5S and 16S rRNAs have been used for the phylogenetic analysis of natural microbial populations. Dr. Norman Pace, an ONR contractor at Indiana University has made major contributions to the development of rRNA probes for the analysis of natural microbial populations (43). The 5S rRNA from mixed natural population can be isolated and species-specific molecules, sorted by high-resolution gel electrophoresis, can then be sequenced. Phylogenetic affinity of organisms are made upon hybridization analysis using 5S rRNA sequences from existing reference files. 5S rRNA has been used to study several natural populations from such locations as Octopus Spring, in Yellowstone National Park; the Chino Mine pond in New Mexico and submarine hydrothermal communities (43).

The 5s rRNA analysis of the microbial population of Octopus Spring reveal the presence of a complex community with three dominant members, two eubacterial species distantly related to *Thermus* spp., and one representive of the archaebacteria. The analysis of 5S rRNA from Chino Mine leaching pond reveals the major microbial population as *Thiobacillus ferrooxidans.*

Dr. Pace continues to devise methods for characterizing natural microbial populations without the necessity for cultivation. He is preparing recombinant DNA libraries of open ocean picoplankton 16S rRNA genes to establish phylogenetic affinities. Dr. Pace has developed a "mixed" kingdom" hybridization probe to be used in the identification and phylogenetic analysis of 16S rDNA picoplankton clones. This probe is a mixture of 16S rRNAs derived from a representative of each primary kingdom, i.e., eukaryote, *Dictyostelium discoideum;* eubacteria, *Escherichia coli;* and archaebacteria, *Sulfolobus solfataricus.* In addition Dr. Pace's laboratory has been able to use group-specific oligodeoxynucleotide probes for identification of the phylogenetic relationship of single microbial cells (25).

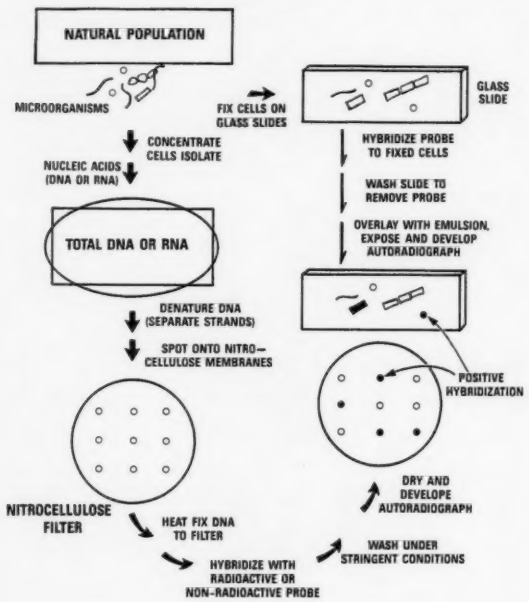
The radio labeled probes specifically hybridized to 16s rRNAs from the targeted kingdom. By microautoradiography, this technique permitted the phylogenetic identification of single cells microscopically. These probes make it possible to count directly, microscopically, the numbers of representative phylogenetic groups in natural populations. As noted by Dr. Pace, radioactive probes are not well suited as single cell probes, since they require microautoradiographic detection which in general is a tedious process. Recently he and his colleagues have developed fluorescent-labeled oligonucleotide probes. The probes are oligonucleotides complementary to the 16S rRNA in the cells. The probes are labeled with fluorescein or rhodamine. Cells from a natural

sample are fixed onto a slide and lysed, after which, the fluroescent-labeled probe mixture (i.e., eubacterial and eukaryotic probes) is applied to the slide. The eukaryotic probe binds only to the eucaryotic cells (i.e., yeast, *Saccharomyces cerevisiae*) while the eubacterial probe binds to the eubacterium, *Bacillus megaterium*.

The use of rRNA to analyze symbiotic relationships in the marine environment might well provide a means to identify unambiguously symbionts and their specific hosts. By providing some details about the phylogenetic affinities of symbionts, this molecular approach will give clues regarding the metabolism and physiology of these organisms. This information is pertinent to our ability to culture marine microorganisms that are presently nonculturable from natural populations.

Figure 5

Probing of natural populations by using hybridization.



Symbiosis at the Hydrothermal Vents

As mentioned earlier hydrothermal vents, represent a complex ecosystem in which dense populations of chemolithotrophic bacteria support rich animal communities (29). These vents are found along ridges at the bottom of the ocean where the earth's crustal plates are spreading apart. Each ecosystem depends on the presence of primary producers: autotrophic organisms, researchers have focused on the identification of the primary producers at the vents. Autotrophic organisms are "self-feeding" in that they are capable of utilizing carbon dioxide as the sole source of carbon and use either light (phototrophs) or chemical oxidation-reduction reactions as a primary source of energy. Because light could not provide the energy for autotrophy at the vents, the basis of life had to be chemolithotrophic (organisms that can oxidize simple inorganic electron donors to obtain energy). The first clue as to the identity of the vent primary producers came with the discovery of high levels of hydrogen sulfide in these underwater hot springs. This is an energy-rich compound which is highly toxic to most life forms. Also found in the vicinity of the hydrothermal vents were Chemoautotrophic oxidizing bacteria. One dominant vent animal is the tube worm, *Riftia pachyptila*. This organism is a closed sac; it has this no mouth or digestive system. Microscopic examination and biochemical studies of the trophosome of *Rirtia* revealed it was colonized by vast numbers of sulfur-oxidizing bacteria (6,21). These studies provided the first evidence that the sulfur-oxidizing bacteria were functional endosymbionts of the tube worms. As a result of this endosymbiosis, the bacteria provide reduced carbon to the worms; and in return the worms provide the bacteria with oxygen, carbon dioxide and hydrogen sulfide.

The use of 5S rRNA sequence has revealed natural population diversity of microorganisms in submarine hydrothermal vent systems (43). These analyses have shown that only a single procaryotic 5S rRNA was associated with a particular host. Therefore, it was concluded that each animal was associated with a clonal population of a single bacterial symbiont. Using the 5S rRNA sequence collection available, then it was found that the closest relative of the symbionts among the free-living sulfur-oxidizing chemoautotrophs was *Thiomicrosipria pelophila* and a Thiomicrospira-like hydrothermal vent isolate, L-12 (45).

Recent evidence indicates that sulfide-based bacterial endosymbiosis is not the only source of primary production at hydrothermal vents. Childress and associates (8) have described a methane based symbiosis between a marine mussel (family Mytilidae) and bacteria. The consumption of methane is limited to the gills of the mussel which harbor intra-cellular bacteria capable of oxidizing methane.

Drs. Fisher and Childress, ONR contractors, currently are investigating the physiological and genetic integration between a marine mussel and its methanotrophic symbionts from the hydrocarbon seeps in the Gulf of Mexico. Their aim is to understand the role symbionts have in meeting the nutritional carbon and nitrogen requirement of the host mussel. Presently, very little is known about the transfer of carbon or nitrogenous metabolities between the host mussel and its symbiotic partner. Radioactive trace experiments using labeled methane (only the symbionts can incorporate methane) will allow monitoring of the flux of organic carbon exchanged by the host mussel and the methanotrophic symbionts.

Additional effort is directed toward evaluating the possible route of nitrogen transfer during symbiosis. These researchers also have initiated experiments to determine the extent of genetic integration between the host and symbionts. It is of particular interest to determine the molecular basis and to what extent symbiont proteins are synthesized on the host ribosomes. A comparison of the genome size of symbiotic and free living methanotrophs is currently being undertaken to get an estimate of the possible loss of genetic material by the symbionts.

Endosymbioses between marine invertebrates and chemolithoautotrophic bacteria appear to represent nutritional symbioses where the respiratory bacteria supply a significant portion of the carbon demand of the host. One problem which greatly hinders progress in the study of these marine endosymbionts is the inability to culture the symbiot bacteria. ONR contractor, Dr. Horst Felbeck, at Scripps Institution of Oceanography is applying 16S rRNA sequencing techniques to characterize marine endosymbionts without culturing them. Sequence data will provide a basis for comparison of symbiont and host phylogeny so as to determine the presence of free-living forms of the symbionts and their "free-living" form can be used to establish the presence of host factors in tissues of the host which control the endosymbiosis.

Many facets of the molecular biology of symbiosis between various organisms remains unknown. The specific events which lead to the recognition of symbiotic partners, the establishment of symbiosis, and the molecular events responsible for the maintenance of the symbiotic relationship are active areas of research. Some of the answers to questions regarding the specificity of selection of symbionts by host might well be supplied by the research of ONR contractor Dr. Robert Trench at the Marine Science Institute, University of California at Santa Barbara, who is investigating the genetic basic of specificity in dinoflagellate-invertebrate symbioses.

Dr. Trench believes the associations between marine invertebrates and microalgae are not random. Instead, there appears to be specificity in the microalgae partner for a functional symbiosis to be established. He is examining the mechanism(s) that bring this specificity. The molecular basis of recognition in symbiosis between microalgae and invertebrates is poorly understood. Dr. Trench hypothesizes that both the initial interactions and the subsequent intracellular interactions between algae and host cells are based on molecular phenomena occurring between animal cell membranes and the surface of the algae. An extensive review of the literature on cellular recognition clearly indicates glycoproteins have a major role in a variety of intercellular recognition processes. The cell wall of the symbiotic algae contains a glycoprotein (53,54) which Dr. Trench plans to isolate, purify and characterize. In particular, Dr. Trench has identified 3 species of *Symbiodinium (S. microadriaticum, S. kawagutii* and *S. microadriaticum)* which are either rejected, accepted and subsequently rejected, or accepted and established as a successful symbiosis. It appears, that at least initial recognition may involve a surface gylcoprotein.

Antibodies produced against the native glycoproteins and its purified apoprotein should help in the identification of the specific components responsible for the specificity of recognition in the selection of algal symbionts.

Genetic Regulation of Bioluminescent Symbiosis

As discussed above a variety of mutualistic associations between marine bacteria and specific hosts exist in the marine environment. However, very little is understood at the molecular level about many of the marine symbiotic systems. Recently a great deal of effort has been devoted to investigating the bioluminescent symbiotic system between *Vibrio fischeri* and the monocentrid (pinecone) fish. The fish are known to maintain pure cultures of *V. fischeri* in specific light organs of the lower jaw and are dependent on the bioluminescence produced by the bacteria for various behavioral activities. How luminescence is regulated by *V. fischeri* as an essential symbiotic function is presently being researched by ONR contractor Dr. Paul Dunlap at New Mexico State University. This system serves as a model for the investigation of the molecular genetics and regulation of symbiosis-specific bacterial genes.

Bacterial luciferase catalyzes the light emission reactions in *V. fischeri*. This enzyme is a mixed function oxidase which uses a long-chain fatty aldehyde and reduced flavin monoucleotide along with molecular oxygen as substrates. The products of the reaction are an oxidized flavin monoucleotide, light, water and a long-chain fatty acid (40). Induction of the *lux* genes is mediated by the concentration of a species-specific compound referred to as an "autoinducer". Transcription of the *lux* operon is dependent upon the accumulation of a critical concentration of the autoinducer

(41,46,39,32). The autoinducer of *V. fischeri* has been identified as homoserine lactone (17) which, along with the *luxR* gene product, activates transcription of the *lux* operon. The *luxI* gene appears to be autoregulated i.e., controlled by its gene product, the autoinducer. Recently it has been shown that cAMP and the cAMP receptor protein (CRP) are required for normal autoinduction of the cloned *V. fischeri lux* genes in *E. coli* (16).

The luminescence *(lux)* system of *V. fischeri* has recently been cloned and isolated as a 9 Kb fragment of DNA that contains the structural and regulatory genes necessary for expression of luminescence. The *lux* operon is composed of a leftward transcribed gene, *luxR* which encodes an autoinducer receptor protein and a central regulatory region that contains promoters and binding sites for regulatory proteins and the six rightward transcribed structural genes for luminescence enzymes. The two subunit polypeptides of luciferase are coded by *luxA* and *luxB.* The *luxC, luxD* and *luxE* genes are required for production of the aldehyde substrate for luciferase.

Studies using gene fusions between *lacZ* and either *luxC* or *luxR* indicate that cAMP and CRP activate transcription of *luxR.* Current results in Dr. Dunlap's laboratory indicate *lux* gene expression may well be controlled at the transcriptional level by two different activators, the CRP and the autoinducer receptor protein. Presently, very little is known about the structure of promoters in marine bacteria. A knowledge of the *lux* promoter structure is essential to our understanding of the molecular details of *lux* gene regulation. Recently, the nucleotide sequence of the *luxA* and *luxB* genes of *Vibrio fischeri* has been determined (22). The *luxA* and *luxB* genes are 1065 and 981 nucleotides in length (355 and 327 amino acids), respectively. The genes have also been cloned and sequenced in *Vibrio harveyi* (10,30). There is about 63% homology between the *luxA* genes of *V. fischeri* and *V. harveyi* and 52% homology between the *luxB* genes of these bioluminescent marine bacteria.

Presently, Dr. Dunlap's efforts are focused on delineating *lux* promoter structure and function in detail using DNA sequence analysis, *in vitro* mutagenesis, and transcriptional assays. Location and isolation of the *lux* transcriptional control region will be accomplished by using the pKO galK expression vectors (38). Restriction fragments of *V. fischeri* DNA containing portions of the *lux* regulatory region and its flanking sequences are being cloned into the expression vector. Newly inserted promoter sequences will allow colonies to be identified and selected by their growth on indicator plates.

This is a very efficient, simple and qualitative screening process for promoter-positive clones. Detailed analysis of binding domains of RNA polymerase, CRP and the *luxR* gene product are presently being investigated using DNA footprinting experiments. The construction of *V. fischeri* regulatory mutants and mutations of these genes in the expression of the *lux* system.

The molecular analysis of the marine bioluminescent symbiosis i.e., the *lux* gene system, is of biotechnological importance in the development of "reporter genes". These genes provide a means to measure gene expression using light and allows the visualization of transcriptional and translational events as well as the localization of genes to particular domains or cells (49). The development of reporter genes from marine bacteria could provide powerful genetic probes for the simultaneous detection of different genes. This is possible because the frequencies of light emitted from various marine bioluminescent bacteria differ, (luciferase produces blue-green light in *V. harveyi* and yellow-green light in *V. fischeri*) and could provide the means to have probes producing different wave length of light emmission which could be discriminated by convential optical techniques.

Conclusion

This review has highlighted some of the biotechnological approaches taken in the Molecular Biology Program at ONR to investigate the molecular biology of marine microorganisms. Understanding the molecular biology of these organisms and how they interact with their environment is pertinent to our ability to control and exploit them biotechnologically. Much of the basic research supported involves investigations concerned with complex behavorial responses of marine microorganisms; genetic engineering of marine bacteria; novel metabolic pathways; genetic engineering of novel biopolymers, microorganisms existing in extreme environmental habitats; and molecular marine symbiosis. Given the present state of scientific technology and advances in molecular biology the stage is set for harnessing of the marine biologic world.

The fruits of this labor have already permitted us in the last decade to genetically engineer genes and proteins, manipulate molecular regulatory switches, continue to decipher the codes of life and to identify microorganisms of natural marine populations or biofouling communities. Molecular biotechnological advances have made it possible to identify, study and harness novel materials from microorganisms existing in extreme environmental habitats. Techniques have been developed which allow the tagging of specific genes so that their environmental regulatory signals can be detected and studied. Genetic engineering has provided a means for unlocking and harnessing the molecular secrets of interesting biomolecules.

One of the salient features of the biotechnological approach taken to investigate the molecular biology of marine microorganisms is its multidisciplinary nature. This demands a cooperative effort between a number of different scientific disciplines and programs. In many cases there already exist close relationships between the life sciences and chemical and computer engineering technology.

ONR has served to catalyze a revitalization of molecular marine biology. Its programs continue to seek a fundamental understanding of marine organisms and their interactions with each other. Practical biotechnological applications of the advances made in molecular marine biology will provide insights into relevant Naval problems and needs.

Acknowledgments

Drs. Leo Parks, Michael T. Marron, and Randall Alberte are thanked for their discussion and critical evaluation of the manuscript. The secretarial work of Ms. Donna Jennings is greatly appreciated. Thanks is also extended to Drs. Robert Newburgh and Steven Zornetzer for their encouragement and support during the preparation of the manuscript. ONR contractors are thanked for their expert research and cooperation which made this review possible.

Biographies

Dr. Thoyd Melton is an Associate Professor in the Microbiology Department at North Carolina State University, Raleigh, North Carolina. Currently, he serves as a Scientific Officer in the Molecular Biology Program at the Office of Naval Research. Dr. Melton earned a Ph.D. in biology (microbial genetics) from The Johns Hopkins University, Baltimore, Maryland.

CDR John W. Bodnar is a Naval Reserve Officer who served with the Office of Naval Research Detachment in Boston, MA. He is currently an Assistant Professor in the Department of Biology at Northeastern University, Boston, MA. He is a U.S. Naval Academy graduate who served on two attack submarines during his active service. After release from active duty he earned a Ph.D. in biochemistry from Oregon State University and completed a postdoctoral fellowship at Yale University.

References

1. Baross, J.A. and J.W. Deming. 1983 Growth of 'black smoker' bacteria at temperatures of at least 250 C. Nature 303:423-426.

2. Baross, J.A., J.W. Deming and R.R. Becker, 1984. Evidence for microbical growth in high pressure, high-temperature environments. In: *Current Perspectives in Microbial Ecology.* M.J. Klug and C.A. Reddy (eds.), American Society for Microbiology., Washington, D.C., pp. 186-195.

3. Baxter, G. and D. Morse. 1987. G protein and diacyldiglycerol regulate metamorphosis of planktonic molluscan larvae. Proc. Natl. Acad. Sci. USA, 84, 1867-1870.

4. Belas, R., M. Simon, and M. Silverman. 1986. Regulation of Lateral Flagella Gene Transcription in *Vibrio parahaemolyticus*.

5. Blakemore, R. P. 1982. Magnetotactic Bacteria. Ann. Rev. Microbiol. 36, 217-238.

6. Cavanaugh, C. H., S. Gardiner, M.L. Jones, H.W. Jannasch, and J.B. Waterbury. 1981. Prokaryotic cells in the hydrothermal vent tubeworm Riftia pachyptila Jones: possible chemoautrophis symbionts. Science 213:340-342.

7. Charlebois, R.L., W.L. Lam, S.W. Cline and W.F. Doolittle. 1987. Characterization of pHV2 from *Halobacterium volcanii* and its use in demonstrating transformation of an archaebacterium. Proc. Natl. Acad. Sci. USA. 84:8530-8534.

8. Childress, J.J., C.R. Fischer, J.M. Brooks, M.C. Kennicutt II, R. Budugare, A.E. Anderson. 1986. A methanogenic marine molluscan (Bivalvia Mytilidae) symbiosis: mussels fueled by gas. Science 233:1306-1308.

9. Cline, S.W., and W.F. Doolittle. 1987. Efficient transfection of the archaebacterium *Halobacterium halobium*. J. Bacteriol. 169:1341-1344.

10. Cohn, D.H., A.J. Mileham, M. Simon, and K. Nealson: 1985. Nucleotide sequence of the *luxA* gene of *Vibrio harveyi* and the complete amino acid sequence of the alpha subunit of bacterial luciferase. J. Biol. Chem. 260:6139-6146.

11. Cue, D., G.S. Beckler, J.N. Reeve, and J. Konisky. 1985. Structure and divergence of two archaebacterial genes. Proc. Natl. Acad. Sci. USA 82:4204-4211.

12. Daniels, C.J., R. Gupta, and W.F. Doolittle. 1985. Transcription and excision of a large intron in the tRNA-trp gene of archabacterium, *Halobacterium volcanii*. J. Biol. Chem. 260:3132-3134.

13. Daniels, L., N. Belay, B.S. Rajagopal, and P.J. Weimer. 1987. Bacterial Methanogenesis and Growth from C02 with Elemental Iron as the Sole Source of Electrons. Science, 237:509-511.

14. Dennis, P. 1986. Molecular biology of archaebacteria, J. Bacteriol. 168:471-478.

15. Dennis, P., J. Chant, and I. Hui. 1986. Structure and expression of ribosomal RNA genes in archaebacteria, p. 356-362. In L. Leive (ed), *Microbiology, 1986.* American Society for Microbiology, Washington, D.C.

16. Dunlap, P.V. and E.P. Greenberg. 1985. Control of *Vibrio fischeri* luminescence gene expression in Escherichia coli by cyclic AMP and receptor protein. J. Bacteriol. 164:45-50.

17. Eberhard, A., A.L. Burlingame, C. Eberhard, G.L. Kenyon, K.H. Nealson, and N.J. Oppenheimer. 1981. Structural identification of autoinducer of *Photobacterium fischeri* luciferase. Biochemistry 20:244-2449.

18. Edmond, J.M., K.L. von Damm, R.E. McDuff and C.I. Measures. 1982. Nature (London) 297:187-191.

19. Eisenberg, H., and E.J. Wachtel. 1987. Structural studies of halophilic proteins, ribosomes, and organelles of bacteria adapted to extreme salt concentrations. Ann. Rev. Biophys. Chem. 16:69-92.

20. Engebracht, J. and M. Silverman. 1986. Techniques for Cloning and Analyzing Bioluminescence Genes from Marine Bacteria. Meth. Enzymol. 133:83-98.

21. Felback, H., J.J. Childress and G.N. Somero. 1981. Calvin-Benson cycle and sulfide oxidation enzymes in animals from sulfide-rich habitats. Nature 293:291-293.

22. Foran, D.R., and W.M. Browm. 1988. Nucleotide sequence of the *LuxA* and *LuxB* genes of the bioluminescent bacterium *Vibrio fischeri.* Nucleic Acid Res. 16:777.

23. Gupta, R. 1984. *Halobacterium volcanii* tRNA: identification of 41 tRNAs covering all amino acids and the sequence of 33 class 1 tRNAs. J. Biol. Chem. 259:9461-9471.

24. Gupta, R. 1985. Transfer ribonucleic acids of archaebacteria, p. 314-344. In C.R. Woese and R.S. Wolfe (ed.) The Bacteria, vol. B. Archaebacteria. Academic Press, Inc., New York.

25. Giovannoni, S.J., E.F. Delong, G.J. Olsen, and N.R. Pace. 1988 Phylogenetic-group specific oligodeoxynucleotide probes for identification of single microbial cells. J. Bacteriol. 170:720-726.

26. Hofman, J.D., L.C. Schalkwyk, and W.F. Doolittle. 1986. ISH51: a large, degenerate family of insertion sequence-like elements in the genome of the archaebacterium, *Halobacterium volcanii.* Nucleic Acid Res. 14:6893-7000.

27. Hamilton, P., and J. Reeve. 1985. Structure of genes and an insertion element in the methane producing archaebacteria, *Methanobrevibacter smithii.* Mole. Gen. Genet. 200:47-55.

28. Hamilton, P. and J. Reeve. 1986. Molecular biology of archaebacteria. p. 338-348. In L. Leive (ed.), *Microbiology, 1986.* American Society of Microbiology, Washington, D.C.

29. Jannasch, H.W. and C.O. Wirsen. 1979. Chemosynthetic primary production at East Pacific sea floor spreading centes. Bioscience 29:592-598.

30. Johnston, T.C., R.B. Thompson and T.O. Baldwin. 1986. Nucleotide sequence of the *luxB* gene of *Vibro harveyi* and the complete amino acid sequence of the beta-subunit of bacterial luciferase. J. Biol. Chem. 261:4805-4808.

31. Kaine, B. R. Gupta, and C.R. Woese. 1983. Putative introns in tRNA genes of prokaryotes. Proc. Natl. Acad. Sci. USA 80:3309-3312.

32. Kaplan, H.B. and E.P. Greenberg. 1985. Diffusion of autoinducer is involved in regulation of the *Vibrio fischeri* luminescence system. J. Bacteriol. 163:1210-1214.

33. Lonsdale, P. 1977. Clustering of suspension-feeding macrobenthos near abyssal hydrothermal vents at oceanic spreading centers. Deep-Sea Res. 24:857-863.

34. Lonsdale, P.F. J.L. Bischoff, V. M. Burns, M. Kastner and R. E. Sweeney. 1980. A high-temperature hydrothermal deposit on the seabed at a Gulf of California spreading center. Earth Planet. Sci. Lett. 49:411-449.

35. Mann, S. 1988. Molecular Recognition in Biomineralization. Nature (London) 332, 119-124.

36. Matsui, E. 1986. Adhesion of Coatings to Surfaces in Naval Civil Engineering Lab FY1986 IR/IED Report:p. 23.

37. Mevarech, M. and R. Werczberger. 1985. Genetic transfer in *Halobacterium volcanii.* J. Bacteriol. 162:461-462.

38. McKenney, K., H. Shimatake, D. Court, U. Schmeissner, C. Brandy, and M. Rosenberg. 1981. A system to study promoter and terminator signals recognized by *Escherichia coli* RNA polymerase. pp. 383-415, In: J.G. Chirikjian and T. Papas (eds.), Gene Amplication and Analysis. Vol. II., Elsevier, North Holland

39. Nealson, K. H. 1977. Autoinduction of bacterial luciferase. Occurrence mechanism and significance. Arch. Microbiol. 112:73-79.

40. Nealson, K. H. and J.W. Hastings. 1979. Bacterial bioluminescence, its control and ecological significance. Microbiol. Rev. 43:496-518.

41. Nealson, K. H., T. Platt, and J.W. Hastings. 1970. Cellular control of the synthesis and activity of the bacterial luminescent system. J. Bacteriol. 104:313-322.

42. Olsen, G.J., N.R. Pace, M. Nuell, B.P. Kaine, R. Gupta and C.R. Woese. 1985. Nucleotide sequence of the 16S rRNA gene from the thermoacidophilic archaebacterium *Sulfolobus solfataricus.* J. Molec. Evol. 22:301-307.

43. Pace, N.R., D.A. Stahl, D.J. Lane, and J. Olsen. 1986. The analysis of natural microbial populations by ribosomal RNA sequences. Adv. Microb. Ecol. 9:1-55.

44. Pfeifer, F., and M. Bethack. 1985. Genomic Organization in *H halobium:* a 70 kb island of more (AT) rich DNA in the chromosomes. Mol. Gen. Genet. 198:449-455.

45. Ruby, E.G. and H. Jannasch. 1982. Physiological characteristics of *Thiomicrospira* sp. L-12 isolated from deep sea hydrothermal vents. J. Bacteriol. 149:161-165.

46. Ruby, E.G. and K.H. Nealson. 1976. Symbiotic association of *Photobacterium fischeri* with the marine luminous fish *Monocentris iaponica:* a model of symbiosis vased on bacterial studies. Biol. Bull. 151:574-586.

47. Sapienza C., and W.F. Doolittle. 1982. Unusual physical organization of the Halobacterium genome. Nature (London) 295:384-389.

48. Sapienza, C., M.R. Rose and W.F. Doolittle. 1982. High frequency genomic rearrangements involving archaebacterial repeat sequence elements. Nature (London) 295:384-389.

49. Schauer, A.T. 1988. Visualizing gene expression with luciferase fusions. TIBTECH 6:23-27.

50. Simonet, B.R.T. and P.F. Lonsdale. 1982 Hydrothermial petroleum in mineralized mounds at the seabed of Guaymas Basin. Nature 295:198-202.

51. Sment, K.A., and J. Konisky. 1986. Regulated expression of the *Methanococcus voltae hisA* gene. Syst. Appl. Microbiol. 7:90-94.

52. Spiess, F.N., K.C. Macdonald, T. Atwater, R. Ballard, A. Carranza, D. Cordoba, C. Cox, V.M. Diaz Garcia, J. Francheteau, J. Guerrero, J. Hawkins, R. Hayman, R. Hessler, T. Jutean, M. Kastner, R. Larson, B. Luyendyk, J.D. Macdougall, S. Miller, W. Normark, J. Orcutt and C. Rargin. Last Pacific Rise: Hot Springs and Geophysical Experiments. 1980. Science 207:1421-1432.

53. Trench , R.K., R.J. Blank. 1987. Symbiodinium micradriaticum Freudenthal, *S. goreauii* sp. nov., *S. kawagutii* sp. nov., and *S. pilosum sp.* nov.; gymnodinioid dinoflagellate symbionts of marine invertebrates. J. Phycol. 23:469-481.

54. Trench, R.K., N.J. Colley and W.K. Fitt. 1981. Recognition phenomena in symbiosis between marine invertebrates and zooxanthellae: uptake, sequestration and persistence. Ber. Duetsch. Bost. Ges. 94:529-545.

55. Waite, J.H. 1987. Nature's underwater adhesive specialist. Int. J. Adhesion and Adhesives. 7:9-14.

56. Walch, M. and R. Mitchell. 1984. Biological Aspects of Corrosion of Offshore Structues. Naval Research Reviews. 36:13-19.

57. Wick, G.L. Sibold. and A. Bock. 1986. Genes for tRNA and their putative expression signals in *Methanococcus*. Syst. Appl. Microbiol. 7:18-25.

58. Woese, C.R., and G.E. Fox. 1977. Phylogenetic structures of the prokaryotiç domain; the primary kingdoms. Proc. Natl. Acad. Sci. USA 74:5088-5090.

59. Woese, C.R., L.J. Magrum and G.E. Fox. 1978. Archaebacteria. J. Molec. Evol. 11:245-252.

60. Woese, C.R., and G.J. Olsen. 1986. Archaebacterial phylogeney: perspective of the urkingdoms. Syst. Appl. Microbiol. 7:161-177.

61. Worrell, V.E. and R. Werczberger. 1985. Genetic transfer in *Halobacterium volcanii*. J. Bacteriol. 162:461-462.

62. Yang, D., B.P. Kaine, and C.R. Woese. 1985. The phylogene of archaebacteria. Syst. Appl. Microbiol. 6:251-256.

Research Notes

NAVY-SUPPORTED RESEARCH MAY BE KEY TO DOLPHIN SURVIVAL

A citizen concerned about the plight of the Atlantic bottlenose dolphins, which have been beaching themselves along the east coast in recent months, wrote to the Office of Naval Research (ONR), asking why is the Navy so interested in dolphins, and why does it conduct research on these marvels of the deep.

The Navy's response highlighted two major reasons. First, the Navy is particularly interested in the welfare of dolphins, because of the close relationship they have as working partners in the ocean. Secondly, the Navy recognizes the marine mammal as an integral part of the ecosystem. Through carefully guided research programs, the Navy strives to understand the animals' remarkable abilities to accomplish certain tasks, and to develop ways in which the animal can be protected, especially from mammal-related diseases, a relatively unknown scientific research area.

One such research program is that of Dr. Brad Fenwick at Kansas State University, formerly of the College of Veterinary Medicine, University of Florida, where he initiated his research. Fenwick's ONR-sponsored research involves searching for means with which to protect dolphins from various diseases by understanding the interaction and events that alter the natural immune function of normal animal cells. Such knowledge will help man to identify those animals that are likely to develop disease and provide, through applied research, the means to protect them against disease. Fenwick's project is part of the Navy's research in biological systems, studies that broadly cover terrestrial and aquatic life forms.

The value of Navy research in biological systems, especially marine mammal research, is evident in the published literature. To date, some 160 articles and reports on marine bioscience and marine animal behavior are referred to in literature gathered and prepared by the Navy.

Much of this scientific data evolves through contracts with universities where the Navy is studying several areas of marine mammal behavior and disease control. Among these studies are animal vision, hearing, and sonar capabilities; animal nutrition; animal reproduction; animal selection and training; and vaccines and general immunology.

It is the disease control aspect of the Navy's research which is receiving special attention lately, brought on by the mysterious stranding—or beaching—of the Atlantic bottlenose dolphin, *Tursiops truncatus*, along the eastern seaboard. Of the scores of dolphins which have beached themselves, virtually all show evidence of a disease that creates blister-like growths or pockmarks on the animals' skins.

Working toward understanding the cause of the dolphin beachings is a team of scientists led by another ONR-sponsored bioscientist, Dr. J. R. Geraci, from the Ontario Veterinary College, Canada. Geraci's focus is on identifying a bacteria, which he believes appears to have affected the animals and caused them to succumb to pneumonia, cerebral hemorrhage, secondary invasion by fungal organisms, and vascular collapse or shock.

In efforts to isolate the cause, Geraci's team is following four lines of investigation: review the types and amounts of industrial and municipal wastes discharged into mid-Atlantic coastal waters; assess meteorological and oceanic current and temperature patterns (historical and present) that might have affected the flora and fauna of the region, and the distribution pattern of discharged wastes; examine the tissue levels of heavy metals, organochlorines and other contaminants; and, examine tissues for biological toxins developed from alga or planktonic growth.

The shocking reality of the importance of such research is scientists believe perhaps as much as 50% of the nearshore population of the bottlenose dolphins along the east coast have died. Those animals who have recovered are impaired of their strength, no doubt because of the disease.

The health of the animals is also a major concern of Dr. Alvin W. Smith, a professor at College of Veterinary Medicine, Oregon State University, Corvallis, Oregon. Under his ONR-sponsored research, Smith is investigating health problems of marine mammals associated with stress. It is his belief that a biological interaction may be causing the dolphin illnesses, resulting from stress induced immunosuppression or a disease induced stress. This interaction—stress induced disease susceptibility and disease induced stress—is the thrust of his research, which is study of the naturally occurring viruses of marine mammals.

The Navy is particularly in the forefront of the preservation and health of dolphin species, according to Dr. Donald Woodward, scientific officer for the biological intelligence branch at the Office of Naval Research. Currently, ONR is funding nine marine mammal research contracts at colleges and universities. Woodward says the Navy shares its knowledge and technology with civilian industry, academic institutions and other government agencies, thus broadening the basic understanding of marine animals.

Although marine mammal research has been a major concern of the Navy for several decades, it wasn't until 1968 when the first isolation of a virus from a marine mammal was made under ONR sponsorship. Since then, only a handful of other viruses have been isolated, including one reported in 1983, taken from small abnormal lesions on the skin of Atlantic bottlenose dolphins.

Because of the Navy's interest in the welfare of the dolphin and the research being conducted on these magnificent animals, they may survive. With any luck, perhaps the research being conducted by Drs. Fenwick, Geraci, Smith, and others under contract with the Office of Naval Research, will uncover the mysteries of the beached dolphins' disease. Meanwhile, with the Navy's help, the search goes on.

Tech Base Notes

Described below is a synopsis of recent technology accomplishments sponsored by the Office of Naval Technology, which is the Navy research and development (R&D) organization responsible for conducting the exploratory development (6.2 category) for the Navy. Exploratory development programs are conducted in industry, academia and in the Navy's own R&D centers.

Field Dressing For Large Combat Wounds:

A pourable wound covering, that both incorporates a bactericidal activity for wound decontamination and provides a protective matrix for the in-growth of new cells and blood capillaries, has been developed by the Naval Medical Research and Development Command under Office of Naval Technology (ONT) sponsorship. The main component of this dressing is collagen, a major structural protein of tissues and bone. When the dressing is poured into a wound and is warmed to the body temperature, it solidifies into a strong protective gel. Fibroblast cells and blood capillaries required for wound repair migrate into this gel and promote rapid healing. Preliminary clinical trials treating leg ulcers will be started in FY-1989. Complications that have often followed the bacterial infections of inadequately covered, large combat wounds promise to be reduced substantially by this method.

First Functional Neural Computer:

The first known operation of a real neural computer was achieved at the Naval Weapons Center as part of the ongoing Office of Naval Technology sponsored 6.2 program. The neural computer is a multi-layer, analog Very Large Scale Integrated (VLSI) network, consisting of 64 neurons, 2048 synapses and 32 parallel input and output lines respectively. It is fully connected to standard digital computers through banks of Analog to Digital (A to D) and D to A converters. Learning takes place by modifying the weighted synaptic connections between the neurons. The computer uses a learning paradigm based upon an algorithm developed by Professor B. Widrow of Stanford University. The world's first analog VLSI implementation of an artificial neural network with demonstrated capability "to learn" is the forerunner of future systems with military applications such as might be used in adaptive processing and control, automatic tracking, data fusion and object recognition.

Cooling Vests Successffully Tested for Shipboard Use:

Working in hot shipboard environments, especially in the primary engine spaces where no cooling is provided, is a health hazard which requires the rotation of personnel and limits the amount of time a person can safely work. Heat stress can also reduce effectiveness and increase the likelihood of errors.

Under sponsorship of the Office of Naval Technology, the Navy Clothing and Textile Research Facility has conducted a shipboard evaluation of a portable cooling vest that can keep a person comfortable for several hours in hot shipboard environments. Unlike battery-powered portable cooling vests, the vest tested is a passive system incorporating coolant packs which can be removed and refrigerated as needed. The vest is easy to use and requires little maintenance and logistical support beyond that required to freeze the coolant packs.

Testing of this low-cost cooling vest (about $200 per system) took place under actual operational conditions in the Persian Gulf in the summer of 1988. In that area the air temperature can exceed 125° F; the water surface can be 95° F or higher.

The evaluation showed that the vest can give a person about two and one-half hours of cooling between coolant changes, and provides a reduction in heat stress comparable to that of more expensive, battery-powered, liquid cooled vests. The vest's small bulk does not interfere with the performance of the sailors' duties.

As a result of Persian Gulf tests and the high acceptance of the vest by shipboard personnel, the Navy is procuring 11,000 vests to outfit 340 ships. The Navy is also modifying the Physiological Heat Exposure Limit—the established standards for personal cooling—which regulates the time a person can spend in hot environments.

DEPARTMENT OF THE NAVY
OFFICE OF NAVAL RESEARCH
ARLINGTON, VA. 22217

OFFICIAL BUSINESS
PENALTY FOR PRIVATE USE. $300

THIRD-CLASS MAIL
POSTAGE & FEES PAID
USN
PERMIT No. GS-9